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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61L 27/00

A2

(11) International Publication Number: WO 98/51354

(43) International Publication Date: 19 November 1998 (19.11.98)

(21) International Application Number: PCT/US98/09951

(22) International Filing Date: 15 May 1998 (15.05.98)

(30) Priority Data:

60/046,589	15 May 1997 (15.05.97)	US
09/039,107	14 March 1998 (14.03.98)	US

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(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published

Without international search report and to be republished upon receipt of that report.

(54) Title: COMPOSITIONS FOR MORPHOGEN-INDUCED OSTEOGENESIS

(57) Abstract

Disclosed herein are improved osteogenic devices and methods of use thereof for repair of bone and cartilage defects.

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COMPOSITIONS FOR MORPHOGEN-INDUCED OSTEOGENESIS

Field of the Invention

The invention relates to materials and methods for correcting orthopedic defects using osteogenic proteins.

Background of the Invention

Morphogens are able to induce the proliferation and differentiation of progenitor cells into functional bone, cartilage, tendon, and/or ligament tissue. This class of proteins includes members of the family of bone morphogenetic proteins (BMPs) identified by their ability to induce ectopic, endochondral bone morphogenesis. The morphogens, also referred to as, osteogenic proteins generally are classified as a subgroup of the TGF-β superfamily of growth factors (Hogan (1996) Genes & Development 10:1580-1594). Members of the morphogen family of proteins include the mammalian osteogenic protein-1 (OP-1, also known as BMP-7, and the *Drosophila* homolog 60A), osteogenic protein-2 (OP-2, also known as BMP-8), osteogenic protein-3 (OP-3), BMP-2 (also known as BMP-2A or CBMP-2A, and the *Drosophila* homolog DPP), BMP-3, BMP-4 (also known as BMP-2B or CBMP-2B), BMP-5, BMP-6 and its murine homolog Vgr-1, BMP-9, BMP-10, BMP-11, BMP-12, GDF3 (also known as Vgr2), GDF8, GDF9, GDF10, GDF11, GDF12, BMP-13, BMP-14, BMP-15, GDF-5 (also known as CDMP-1 or MP52), GDF-6 (also known as CDMP-2), GDF-7 (also known as CDMP-3), the *Xenopus* homolog Vgl and NODAL, UNIVIN, SCREW, ADMP, and NEURAL.

Members of this family encode secreted polypeptides that share common structural features. The mature form of such proteins results from processing through a "pro-form" to yield a mature polypeptide chain competent to dimerize and containing a carboxy terminal active domain of approximately 97-106 amino acids. All members share a conserved pattern of cysteines in this domain and the active form of these proteins can be either a disulfide-bonded homodimer of a single family member or a heterodimer of two different members (see, e.g., Massague (1990) Annu. Rev. Cell Biol. 6:597; Sampath, et al. (1990) J. Biol. Chem. 265:13198). See also, U.S. 5,011,691; U.S. 5,266,683, Ozkaynak et al. (1990) EMBO J. 9: 2085-2093,

Wharton et al. (1991) PNAS 88:9214-9218), (Ozkaynak (1992) J. Biol. Chem. 267:25220-25227 and U.S. 5,266,683); (Celeste et al. (1991) PNAS 87:9843-9847); (Lyons et al. (1989) PNAS 86:4554-4558). These disclosures describe the amino acid and DNA sequences, as well as the chemical and physical characteristics, of osteogenic proteins. See also, Wozney et al. (1988) Science 242:1528-1534); BMP 9 (WO93/00432, published January 7, 1993); DPP (Padgett et al. (1987) Nature 325:81-84; and Vg-1 (Weeks (1987) Cell 51:861-867).

True osteogenic proteins capable of inducing the above-described cascade of morphogenic events resulting in endochondral bone formation, have now been identified, isolated, and cloned. Whether naturally-occurring or synthetically prepared, these osteogenic factors, when implanted in a mammal in association with a matrix or substrate that allows attachment, proliferation and differentiation of migratory progenitor cells, can induce recruitment of accessible progenitor cells and stimulate their proliferation, thereby inducing differentiation into chondrocytes and osteoblasts, and further inducing differentiation of intermediate cartilage, vascularization, bone formation, remodeling, and, finally, marrow differentiation. Furthermore, numerous practitioners have demonstrated the ability of these osteogenic proteins, when admixed with either naturally-sourced matrix materials such as collagen or synthetically-prepared polymeric matrix materials, to induce bone formation, including endochondral bone formation, under conditions where true replacement bone otherwise would not occur. For example, when combined with a matrix material, these osteogenic proteins induce formation of new bone in large segmental bone defects, spinal fusions, and fractures.

Needs remain for carriers for delivering osteogenic protein to a bone repair locus. Preferred carriers are provided by the present invention.

Summary of the Invention

The present invention provides delivery systems and methods for providing osteogenic protein to bone defect sites. In a preferred emodiment, a delivery system of the invention comprises osteogenic protein in a calcium phosphate matrix. It has now been recognized that use of a calcium phosphate matrix for delivery of osteogenic protein to defect sites in bones that do not undergo active remodeling, especially in short, irregular, or flat bone defect sites, greatly improves the structure of new bone ingrowth. It has also been recognized that providing preferred ratios of calcium phosphate to hydroxyapatite results in improved cosmetic results.

The invention provides, in one aspect, a device for inducing local bone and/or cartilage formation. A preferred device of the invention comprises an osteogenic protein in a calcium phosphate matrix. As contemplated herein, the device preferably comprises osteogenic proteins such as, but not limited to, OP-1, OP-2, BMP-2, BMP-4, BMP-5 and BMP-6. A currently preferred osteogenic protein is OP-1. As used herein, the terms "morphogen", "bone morphogen", "bone morphogenic protein", "BMP", "osteogenic protein" and "osteogenic factor" embrace the class of proteins typified by human osteogenic protein 1 (hOP-1). Nucleotide and amino acid sequences for hOP-1 are provided in Seq. ID Nos. 1 and 2, respectively. OP-1 is merely representative of the TGF-β subclass of true chondrogenic tissue morphogens competent to act as osteogenic proteins, and is not intended to be limiting. Other known, and useful proteins include, BMP-2, BMP-3, BMP-3b, BMP-4, BMP-5, BMP-6, BMP-8, BMP-9, BMP-10, BMP-11, BMP-12, BMP-13, BMP-15, GDF-1, GDF-2, GDF-3, GDF-5, GDF-6, GDF-7, GDF-8, GDF-9, GDF-10, GDF-11, GDF-12, NODAL, UNIVIN, SCREW, ADMP, NEURAL and osteogenically active amino acid variants thereof. In one preferred embodiment, proteins useful in the invention include biologically active species variants of any of these proteins, including conservative amino acid sequence variants, proteins encoded by degenerate nucleotide sequence variants, and osteogenically active proteins sharing the conserved seven cysteine skeleton as defined herein and encoded by a DNA sequence competent to hybridize to a DNA sequence encoding an osteogenic protein disclosed herein, including, without limitation, OP-1, BMP-5, BMP-6, BMP-2, BMP-4 or GDF-5, GDF-6 or GDF-7. In another embodiment, useful osteogenic proteins include those sharing the conserved seven cysteine domain and sharing at least 70% amino acid sequence homology (similarity) within the C-terminal active domain, as defined herein. In another embodiment, useful proteins include those sharing greater than 60% identity in the C-terminal domain. In still another embodiment, useful osteogenic proteins can be defined as osteogenically active proteins having any one of the generic sequences defined herein, including OPX (SEQ ID No: 3) and Generic Sequences 7 and 8 (Seq. ID Nos. 4 and 5), or Generic Sequences 9 and 10 (Seq. ID Nos. 6 and 7).

A calcium phosphate matrix for use in the invention may be supplied in any biocompatible form, and is preferably in the form of hydroxyapatite, tricalcium phosphate, or any other form that is resorbable during new bone growth. Calcium phoshpate may be supplied in the form of a powder, solid blocks, cements, pastes, shaped forms, or any other form that is resorbed, in whole or in part, during new bone ingrowth. Calcium phosphate for use in a device or method of the

invention may be integrated or admixed with other carrier materials, such as collagen or a collagen-based carrier, including polyglycolic acid, polylactic acid, polybutyric acid, polysylicates, and their derivatives, or combinations.

In another aspect, the instant invention provides methods for inducing local bone or cartilage formation, or for repair of bone, cartilage or osteochondral defects. In a preferred embodiment, a method of the invention comprises administering to a bone defect site an osteogenic protein in a calcium phosphate matrix. It has now been discovered that a calcium phosphate matrix is superior to other matrices for repair of defects, especially in short, irregular, or flat bones. Accordingly, in a particularly preferred embodiment, the invention provides a method for inducing new bone growth in a small bone of the head, face, hands, or feet, comprising implanting an osteogenic device in a short, irregular, or flat bone defect site, the osteogenic device comprising an osteogenic protein in a calcium phosphate matrix in an amount sufficient to stimulate new bone growth in the defect site. Methods of the invention are particularly useful for providing augmentation of the bones of the face, such as, for example, the mandible or the maxilla. While a calcium phosphate matrix is useful for reconstruction of any bone, including long bones, methods of the invention are particularly useful in cosmetic surgery procedures, wherein precise bone reconstruction is desirable or required.

Also in a preferred embodiment, the invention provides methods for repairing a bone defect comprising filling the defect with a composition comprising a morphogen in combination with an allograft material, preferably allograft bone chips. In highly preferred embodiments, the invention comprises inserting an impacted allograft into a defect site in order to improve mechanical fixation of the implant.

The instant methods are useful to induce formation of at least endochondral bone, intramembranous bone, and articular cartilage. Bone repair methods of the invention include treatment of both closed and open defects with the above-described improved osteogenic devices. As taught herein, the methods of the instant invention can be practiced using improved devices that are of sufficient volume to fill the defect site, as well as using improved devices that are not. Examples of defects include, but are not limited to, critical size defects, non-critical size defects, non-union fractures, fractures, osteochondral defects, chondral defects and periodontal defects. Further aspects and advantage of the invention will be appreciated upon consideration of the following detailed description thereof.

Brief Description of the Drawings

Figure 1 (panels 1A through 1L) is a tabular alignment of the amino acid sequences of various naturally occurring morphogens with a preferred reference sequence of human OP1, residues 330-431 of SEQ ID NO: 1;

Figure 2 is a tabular presentation of alternative amino acids for "Xaa" positions in generic sequences SEQ ID NOS: 4, 5, and 8 that represent amino acid variations in known morphogens;

Figure 3 is a tabular presentation of alternative amino acids for "Xaa" positions in generic sequences SEQ ID NOS: 6, 7, and 9 that represent amino acid variations in known morphogens;

Figure 4 is a tabular presentation of alternative amino acids for "Xaa" positions in generic sequence SEQ ID NO: 3 that represents amino acid variations in several identified allelic and phylogenetic variants of OP-1 and OP-2.

Detailed Description of Preferred Embodiments

Bones are generally divided into four main types. Long bones are the largest bones of the body (e.g., the femur); short bones are shorter than long bones, and have less prominent ends (e.g., bones of the hands and feet); irregular bones typically possess surfaces that articulate with other bones (e.g., wrist bones); and flat bones typically have plate-like surfaces (e.g., bones of the skull). It has now been discovered that bone repair is greatly facilitated by application to a defect site of an osteogenic protein in a calcium phosphate matrix. It has also been discovered that the use of an optimal ratio of calcium phosphate to osteogenic protein promotes optimal cosmetic bone ingrowth. These effects are most prominent in short, irregular and flat bones, especially where optimal cosmetic results are desirable.

As used herin, "defect", "defect site", or "defect locus", defines an orthopedic structural disruption requiring repair. The defect may occur in a joint, in any bone, including a intramembranous bone, bony, cartilage, tendon, ligament, or an osteochondral defect. A defect can be the result of accident, disease, surgical manipulation, and/or prosthetic failure. In certain embodiments, the defect is a void having a volume incapable of endogenous or spontaneous repair. Such defects are generally twice the diameter of the subject bone and are also called "critical size" defects. For example, in rabbit and monkey segmental defect models, the gap is approximately 1.5 cm and 2.0 cm, respectively. In a canine ulnar defect model, the defect is a 2-4

cm , gap incapable of spontaneous repair. See, e.g., Schmitz *et al.*, Clinical Orthopaedics and Related Research 205:299-308 (1986); Vukicevic *et al.*, in Advanced in Molecular and Cell Biology, Vol. 6, pp. 207-224 (1993)(JAI Press, Inc.). In other embodiments, the defect is a non-critical size segmental defect. Generally, non-critical defects, such as fracture defects, are capable of some spontaneous repair. Application of the devices and formulations described herein can substantially enhance fracture repair, including the rate and quality of newly formed bone. This allows for improved bone healing, especially in compromised individuals such as diabetics, smokers, obese individuals and others who, due to an acquired or congenital condition have a reduced capacity to heal bone fractures. Other defects include osteochondral defect, such as osteochondral plugs. Such a defect traverses the entirety of the overlying cartilage and enters, at least in part, the underlying bony structure. In contrast, a chondral or subchondral defect traverses the overlying cartilage, in part or in whole, respectively, but does not involve the underlying bone. Other orthopedic defects susceptible to repair using the instant invention include, but are not limited to, non-union fractures; bone cavities; tumor resection; fresh fractures (distracted or undistracted); cranial/facial abnormalities; periodontal defects and irregularities; spinal fusions; as well as those defects resulting from diseases such as cancer, arthritis, including osteoarthritis, and other bone degenerative disorders such as osteochondritis dessicans. Still other defects susceptible to repair include joint tissue defects, including defects requiring partial or complete joint reconstruction, including correcting tendon and/or ligamentous tissue defects such as, for example, the anterior, posterior, lateral and medial ligaments of the knee, the patella and achilles tendons, and the like.

In addition to osteogenic proteins, various growth factors, hormones, enzymes, therapeutic compositions, antibiotics, or other bioactive agents also can be contained within an osteogenic device. Thus, various known growth factors such as EGF, PDGF, IGF, FGF, TGF-a , and TGF-β can be combined with an osteogenic device and be delivered to the defect locus. An osteogenic device also can be used to deliver chemotherapeutic agents, insulin, enzymes, enzyme inhibitors and/or chemoattractant/chemotactic factors.

The means for making and using the methods, implants and devices of the invention, as well as other material aspects concerning their nature and utility, including how to make and how to use the subject matter claimed, will be further understood from the following, which constitutes the best mode currently contemplated for practicing the invention. It will be appreciated that the

invention is not limited to such exemplary work or to the specific details set forth in these examples.

I. PROTEIN CONSIDERATIONS

A. Biochemical, Structural and Functional Properties of Bone Morphogenic Proteins

In its mature, native form, natural-sourced osteogenic protein is a glycosylated dimer, typically having an apparent molecular weight of about 30-36 kDa as determined by SDS-PAGE. When reduced, the 30 kDa protein gives rise to two glycosylated peptide subunits having apparent molecular weights of about 16 kDa and 18 kDa. In the reduced state, the protein has no detectable osteogenic activity. The unglycosylated protein, which also has osteogenic activity, has an apparent molecular weight of about 27 kDa. When reduced, the 27 kDa protein gives rise to two unglycosylated polypeptide chains, having molecular weights of about 14 kDa to 16 kDa. Typically, the naturally occurring osteogenic proteins are translated as a precursor, having an N-terminal signal peptide sequence typically less than about 30 residues, followed by a "pro" domain that is cleaved to yield the mature C-terminal domain. The signal peptide is cleaved rapidly upon translation, at a cleavage site that can be predicted in a given sequence using the method of Von Heijne, NUCLEIC ACIDS RESEARCH *14*: 4683-4691 (1986). The pro domain typically is about three times larger than the fully processed mature C-terminal domain.

Osteogenic proteins useful herein include any known naturally-occurring native proteins including allelic, phylogenetic counterpart and other variants thereof, whether naturally-occurring or biosynthetically produced (*e.g.*, including "muteins" or "mutant proteins"), as well as new, osteogenically active members of the general morphogenic family of proteins.

Particularly useful sequences include those comprising the C-terminal 96 or 102 amino acid sequences of DPP (from Drosophila), Vgl (from Xenopus), Vgr-1 (from mouse), the OP-1 and OP-2 proteins (*see* U.S. Patent No. 5,011,691 and Oppermann *et al.*, as well as the proteins referred to as BMP2, BMP3, BMP4 (see WO88/00205, U.S. Patent No. 5,013,649 and WO91/18098), BMP5 and BMP6 (see WO90/11366, PCT/US90/01630), BMP8 and BMP9. Other proteins useful in the practice of the invention include active forms of OP1, OP2, OP3, BMP2, BMP3, BMP4, BMP5, BMP6, BMP9, GDF-5, GDF-6, GDF-7, DPP, Vg1, Vgr, 60A protein, GDF-1, GDF-3, GDF-5, GDF-6, GDF-7, BMP10, BMP11, BMP13, BMP15, UNIVIN, NODAL, SCREW, ADMP or NURAL and amino acid sequence variants thereof. In one currently preferred embodiment, osteogenic protein include any one of: OP1, OP2, OP3, BMP2,

BMP4, BMP5, BMP6, BMP9, and amino acid sequence variants and homologs thereof, including species homologs, thereof. Publications disclosing OP-1 and OP-2 sequences, as well as their chemical and physical properties, include U.S. Patent Nos. 5,011,691 and 5,266,683, incorporated by reference herein.

In preferred embodiments, morphogens for use in methods of the invention include proteins having at least 70% homology with the amino acid sequence of the C-terminal seven-cysteine skeleton of human OP-1, SEQ ID NO: 2, and having the ability to induce endochondral bone formation in the Reddi and Sampath assay described herein. Compounds that meet these requirements are considered functionally equivalent to a known response morphogen. To determine whether a candidate amino acid sequence is functionally equivalent to a reference morphogen, the candidate sequence and the reference sequence are aligned. The first step for performing an alignment is to use an alignment tool, such as the dynamic programming algorithm described in Needleman *et al.*, J. MOL. BIOL. *48*: 443 (1970), and the Align Program, a commercial software package produced by DNAstar, Inc. the teachings of which are incorporated by reference herein. After the initial alignment is made, it is then refined by comparison to a multiple sequence alignment of a family of related proteins, such as those shown in FIG. 1A through 1M, which is a multiple sequence alignment of a family of known morphogens, including hOP-1. Once the alignment between the candidate and reference sequences is made and refined, a percent homology score is calculated. The individual amino acids of each sequence are compared sequentially according to their similarity to each other. Similarity factors include similar size, shape and electrical charge. One particularly preferred method of determining amino acid similarities is the PAM250 matrix described in Dayhoff *et al.*, 5 ATLAS OF PROTEIN SEQUENCE AND STRUCTURE 345-352 (1978 & Supp.), incorporated by reference herein. A similarity score is first calculated as the sum of the aligned pairwise amino acid similarity scores. Insertions and deletions are ignored for the purposes of percent homology and identity. Accordingly, gap penalties are not used in this calculation. The raw score is then normalized by dividing it by the geometric mean of the scores of the candidate compound and the seven cysteine skeleton of hOP-1. The geometric mean is the square root of the product of these scores. The normalized raw score is the percent homology.

In an alternative preferred embodiment, a functionally-equivalent morphogen sequence shares at least 60% amino acid identity with a reference sequence. That is, any 60% of the aligned amino acids are identical to the corresponding amino acids in the reference sequence. Any

one or more of the naturally-occurring or biosynthetic morphogens disclosed herein may be used as a reference sequence to determine whether a candidate sequence falls within the morphogen family. In a preferred embodiment, the reference sequence is the C-terminal seven-cysteine skeleton sequence of human OP-1 as shown in SEQ ID NO: 2. Examples of conservative substitutions for use in the above calculations include the substitution of one amino acid for another with similar characteristics, *e.g.*, substitutions within the following groups are well-known: (a) valine, glycine; (b) glycine, alanine; (c) valine, isoleucine, leucine; (d) aspartic acid, glutamic acid; (e) asparagine, glutamine; (f) serine, threonine; (g) lysine, arginine, methionine; and (h) phenylalanine, tyrosine. The term "conservative variant" or "conservative variation" also includes the use of a substituted amino acid in place of an unsubstituted parent amino acid in a given polypeptide chain, provided that antibodies having binding specificity for the resulting substituted polypeptide chain also have binding specificity (*i.e.*, "crossreact" or "immunoreact" with) the unsubstituted or parent polypeptide.

In a preferred embodiment, morphogens useful in the present invention are defined by a generic amino acid sequence that represents variations in known morphogens. For example, SEQ ID NOS: 4 and 5 encompass observed variations between preferred morphogens, including OP-1, OP-2, OP-3, CBMP-2A, CBMP-2B, BMP-3, 60A, DPP, Vg1, BMP-5, BMP-6, Vgr-1, and GDF-1. SEQ ID NO: 5 includes all of SEQ ID NO: 4, and also includes at its N-terminus the five amino acid sequence of SEQ ID NO: 8. The generic sequences include both the amino acid identity shared by these sequences in the C-terminal domain, defined by the six- and seven-cysteine skeletons (SEQ ID NOS: 4 and 5, respectively), and alternative amino acids for variable positions within the sequence. Positions that allow for alternative amino acids are represented by "Xaa". FIG. 3 shows the alternative amino acids for each "Xaa" position in SEQ ID NOS: 4, 5 and 8. For example, referring to SEQ ID NO: 5 and FIG. 3, the "Xaa" at position 2 may be a tyrosine or a lysine. The generic sequences provide an appropriate cysteine skeleton for inter- or intramolecular disulfide bonding, and contain certain critical amino acids likely to influence the tertiary structure of the proteins. In addition, the "Xaa" at position 36 in SEQ ID NO: 4, or at position 41 in SEQ ID NO: 5, may be an additional cysteine, thereby encompassing the morphogenically-active sequences of OP-2 and OP-3.

In another embodiment, useful morphogens include those defined by SEQ ID NOS: 6 or 7, which are composite amino acid sequences of the following morphogens: human OP-1, human OP-2, human OP-3, human BMP-2, human BMP-3, human BMP-4, human BMP-5, human

BMP-6, human BMP-8, human BMP-9, human BMP-10, human BMP-11, *Drosophila* 60A, Xenopus Vg-1, sea urchin UNIVIN, human CDMP-1 (mouse GDF-5), human CDMP-2 (mouse GDF-6, human BMP-13), human CDMP-3 (mouse GDF-7, human BMP-12), mouse GDF-3, human GDF-1, mouse GDF-1, chicken DORSALIN, *Drosophila* dpp, *Drosophila* SCREW, mouse NODAL, mouse GDF-8, human GDF-8, mouse GDF-9, mouse GDF-10, human GDF-11, mouse GDF-11, human BMP-15, and rat BMP-3b. SEQ ID NO: 7 includes all of SEQ ID NO: 6 and also includes at its N-terminus the five amino acid sequence of SEQ ID NO: 9. SEQ ID NO: 6 accommodates the C-terminal six-cysteine skeleton, and SEQ ID NO: 7 accommodates the seven-cysteine skeleton. Positions that allow for alternative amino acids are represented by "Xaa". FIG. 4 shows the alternative amino acids for each "Xaa" position in SEQ ID NOS: 6, 7 and 9.

As noted above, certain preferred morphogen sequences useful in this invention have greater than 60% identity, preferably greater than 65% identity, with the amino acid sequence defining the preferred reference sequence of hOP-1. These particularly preferred sequences include allelic and phylogenetic variants of the OP-1 and OP-2 proteins, including the *Drosophila* 60A protein, as well as the closely related proteins BMP-5, BMP-6 and Vgr-1. Accordingly, in certain particularly preferred embodiments, useful morphogens include proteins comprising the generic amino acid sequence SEQ ID NO: 3 (referred to herein as "OPX"), which defines the seven-cysteine skeleton and accommodates the homologies between several identified variants of OP-1 and OP-2. Positions that allow for alternative amino acids are represented by "Xaa". FIG. 5 shows the alternative amino acids for each "Xaa" position in SEQ ID NO: 3.

In still another preferred embodiment, useful morphogens include those having an amino acid sequence encoded by a polynucleotide that hybridizes under high stringency conditions with DNA or RNA encoding a reference morphogen. Standard stringency conditions are well characterized in standard molecular biology texts. *See generally* MOLECULAR CLONING A LABORATORY MANUAL, (Sambrook *et al.*, eds., 1989); DNA CLONING, Vol. I & II (D.N. Glover ed., 1985); OLIGONUCLEOTIDE SYNTHESIS (M.J. Gait ed., 1984); NUCLEIC ACID HYBRIDIZATION (B. D. Hames & S.J. Higgins eds. 1984); B. Perbal, A PRACTICAL GUIDE TO MOLECULAR CLONING (1984).

In another embodiment, morphogens useful in the invention include the soluble complex form comprising a mature morphogen dimer linked to a morphogen pro domain or a solubility-enhancing fragment thereof. A solubility-enhancing fragment is any N-terminal or

C-terminal fragment of a morphogen pro domain that forms a complex with the mature morphogen dimer and increases the solubility of the morphogen dimer. Preferably, the soluble complex comprises a morphogen dimer and two pro domain peptides. Morphogen soluble complex is described in published application WO 94/03600, incorporated by reference herein.

In yet another embodiment, useful morphogens include biologically active biosynthetic constructs, including novel biosynthetic morphogens and chimeric proteins designed using sequences from two or more known morphogens. *See* U.S. Patent No. 5,011,691, incorporated by reference herein (*e.g.*, COP-1, COP-3, COP-4, COP-5, COP-7, and COP-16).

II. FORMULATION AND DELIVERY CONSIDERATIONS

A. General Considerations

Devices of the invention can be formulated using routine methods. All that is required is determination of the desired final concentration of osteogenic protein per device, keeping in mind that the delivered volume of device can be, but is not necessarily required to be, less than the volume at the defect site. Useful formulation methodologies include lyophilization of solubilized protein onto a calcium phosphate matrix. Useful protein solubilization solutions include acidic ethanol, urea, acidic buffers, and acetonitrile/trifluoroacetic acid solutions, and the like. See, for example, U.S. 5,266,683. The desired final concentration of protein will depend on the specific activity of the protein as well as the type, volume, and/or anatomical location of the defect. Proteins having lower specific activity also can be used to advantage. Additionally, the desired final concentration of protein can depend on the age, sex and/or overall health of the recipient. Typically, for a critical size bone segmental defect approximately at least 2.5 cm in length, 0.5-1.75 mg osteogenic protein has been observed using the standard device to induce bone formation sufficient to repair the gap. In the case of a non-critical size defect or a fresh fracture, approximately 0.1-0.5 mg protein has been observed using the standard osteogenic device to repair the defect. Optimization of dosages requires no more than routine experimentation and is within the skill level of one of ordinary skill in the art.

Osteogenic devices and formulations are readily sterilized using standard procedures prior to implantation. For example, proteins conveniently can be filter-sterilized, e.g., using a 0.22 micron filter. Matrix and/or carrier materials can be sterilized by exposure to chemicals, heat, or ionizing radiation. In addition, osteogenic devices and formulations can be terminally sterilized to

a sterility assurance level of 10^{-6} by exposure to ionizing radiation, for example, gamma or electron beam radiation. Useful dose ranges include within the range of about 0.5-4.0 megarads, preferably 2.0-3.5 megarads. See, for example, USSN 08/478,452 filed June 7, 1995, or WO 96/40297.

Practice of the invention will be still more fully understood from the following examples, which are presented herein for illustration only and should not be construed as limiting the invention in any way.

III. BIOASSAY

A. Bioassay of Osteogenic Activity: Endochondral Bone Formation and Related Properties

The art-recognized bioassay for bone induction described by Sampath and Reddi (Proc. Natl. Acad. Sci. USA (1983) 80:6591-6595) and US Pat. No. 4,968,590, the disclosures of which are incorporated by reference herein, are useful to establish the efficacy of a given device or formulation. Briefly, the assay consists of depositing test samples in subcutaneous sites in recipient rats under ether anesthesia. A vertical incision (1 cm) is made under sterile conditions in the skin over the thoracic region, and a pocket is prepared by blunt dissection. In certain circumstances, approximately 25 mg of the test sample is implanted deep into the pocket and the incision is closed with a metallic skin clip. The heterotropic site allows for the study of bone induction without the possible ambiguities resulting from the use of orthotopic sites.

The sequential cellular reactions occurring at the heterotropic site are complex. The multistep cascade of endochondral bone formation includes: binding of fibrin and fibronectin to implanted matrix, chemotaxis of cells, proliferation of fibroblasts, differentiation into chondroblasts, cartilage formation, vascular invasion, bone formation, remodeling, and bone marrow differentiation.

Successful implants exhibit a controlled progression through the stages of protein-induced endochondral bone development including: (1) transient infiltration by polymorphonuclear leukocytes on about day one; (2) mesenchymal cell migration and proliferation on about days two and three; (3) chondrocyte appearance on about days five and six; (4) cartilage matrix formation on about day seven; (5) cartilage calcification on about day eight; (6) vascular invasion, appearance of osteoblasts, and formation of new bone on about days nine and ten; (7) appearance

of osteoblastic and bone remodeling on about days twelve to eighteen; and (8) hematopoietic bone marrow differentiation in the ossicle on about day twenty-one. The timecourse of this process varies according to the matrix

Histological sectioning and staining is preferred to determine the extent of osteogenesis in the implants. Staining with toluidine blue or hemotoxylin/eosin clearly demonstrates the ultimate development of endochondral bone. Twelve day bioassays are sufficient to determine whether bone inducing activity is associated with the test sample.

Additionally, alkaline phosphatase activity can be used as a marker for osteogenesis. The enzyme activity can be determined spectrophotometrically after homogenization of the excised test material. The activity peaks at 9-10 days *in vivo* and thereafter slowly declines. Samples showing no bone development by histology should have no alkaline phosphatase activity under these assay conditions. The assay is useful for quantitation and obtaining an estimate of bone formation very quickly after the test samples are removed from the rat. For example, samples containing osteogenic protein at several levels of purity have been tested to determine the most effective dose/purity level, in order to seek a formulation which could be produced on an industrial scale. The results as measured by alkaline phosphatase activity level and histological evaluation can be represented as "bone forming units". One bone forming unit represents the amount of protein that is needed for half maximal bone forming activity on day 12. Additionally, dose curves can be constructed for bone inducing activity *in vivo* at each step of a purification scheme by assaying various concentrations of protein. Accordingly, the skilled artisan can construct representative dose curves using only routine experimentation.

B. Methods of Using a Hydroxyapatite Matrix for Delivery of Osteogenic Protein

An osteogenic device for use in methods of the present invention may comprise any combination of materials suitable to simulate bone growth. Ideally, such materials comprise a biocompatible matrix, implanted at the defect site, upon which new bone growth occurs; an osteogenic protein to stimulate optimal bone growth; and a concentration of calcium phosphate (*e.g.*, hydroxyapatite) to modulate uniform ingrowth. For example, a preferred osteogenic device for use in methods according to the present invention comprises a bovine bone collagen matrix, a ceramic matrix, or a ceramic-collagen composite matrix. The matrix ideally is absorbed into new bone as bone formation takes place. The contents of the matrix may be varied in order to suit a

desired clinical application. For example, the source of the matrix may be varied, as may the osteogenic protein used. Numerous sources of bone matrix (*e.g.*, bovine bone, human bone, collagen, and composites) are known. *See, e.g.*, U.S. Patent Nos. 4,975,526, and 5,354,557,each of which is incorporated by reference herein.

Hydroxyapatite is made by reacting Tetracalcium phosphate and dicalcium phosphate anhydrous or dicalcium phosphate dihydrate in aqueous solution. Hydration of the reactants causes the cement to harden within about thirty minutes to form a microcrystalline lattice. Hydroxyapatite alone will be converted to bone when implanted in physical contact with existing bone near a defect site. However, when osteogenic protein is added, new bone growth, with concomitant osteoconversion of hydroxyapatite, occurs even when there is no physical contact with existing bone. Osteogenic protein generally facilitates the osetoresein on hyroxyapatite-based implants. Methods for preparing hydroxyapatite are reported, *inter* alia, in U.S. Patent Nos. Re. 33,161 and Re. 33,221, each of which is incorporated by reference herein.

Improved methods according to the present invention are especially useful in the repair of craniofacial damage or defects, or in other cosmetic surgery applications in which a uniform appearance of new bone is desired. For example, maxillary and mandibular atrophy is a recurrent problem in maxillofacial surgery. It is often difficult to obtain both the required functional results and to preserve aesthetic quality. As shown below, the amount and quality of bone formation in these bones maybe affected by the choice of matrix.

The effects of hydroxyapatite on osteogenic protein induction of new bone growth were analyzed in a mandibular augmentation procedure. Bilateral pouches were created between the lateral aspect of the mandible and the masseteric muscle in 30 male Wistar rats. The bone surface was penetrated to induce bleeding, and one of six different carriers, each containing recombinant OP-1, were delivered to the defect site. The first two carriers were commercially-available hydroxyapatite matrices (Algipore® and Bio-Oss®). Those were compared with commercially available preparations consisting of silicon granules (Ionogran®), bovine bone matrix (Osteovit®), or a collagen bone matrix. In each case, about 70 μg human recombinant OP-1 in acetate buffer was applied to the matrix. The contralateral side was used as a control in which the matrix contained only buffer.

Sequential post-operative labeling of the implant with fluorochromes was performed to enable visualization of bone ingrowth. The animals then were sacrificed at post-operative day 50, and undecalcified ground sections were processed for microradiography, fluorescent light evaluation, and histo-morphometry. Among the variables assessed were height of augmentation and amount of newly-formed bone. Only negligible bone apposition to the underlying mandibular bone was observed in control sites. While augmentation was observed in all OP-1 implanted sites, significant variations in structure and amount of newly-formed bone were found, depending on the matrix that was used. Results are presented in the table below. Mean augmentation height is provided in mm +/- SD. Bone density was determined as the area of newly-formed mineralized bone per total area of augmentation on 3 microradiographs from each specimen.

Matrix	Mean Augmentation Height (mm)	Bone Density
Algipore®	4.1 ± 0.8	51%
Bio-Oss® granules	5.2 ± 1.3	27%
Bio-Oss Spongiossa block®	5.1 ± 0.9	22%
Osteovit®	4.9 ± 1.2	42%
Ionogran®	3.2 ± 0.7	9.0%
Collagen Bone Matrix®	1.0 ± 0.9	84%

As shown in the table, augmentation height was significantly lower when either Ionogran® or collagen bone matrix was used as the matrix. The optimal combination of a desirable augmentation height and optimal density for cosmetic purposes was observed only in cases in which a hydroxyapatite matrix was used.

IV. FORMULATION OF OP-1/HA COMPOSITE DEVICES

A rat subcutaneous model was used to evaluate the clinical effectiveness of osteogenic protein/hydroxyapatite composite devices containing an optimal ratio of osteogenic protein to hydroxyapatite. A preferred osteogenic protein for use in methods of the invention is the osteogenic protein, OP-1. However, any osteogenic protein may be used in the manner taught herein.

Hydroxyapatite-based matrix formulations may take numerous forms. For example, they may be liquid solutions (e.g. 5% lactose, 20mM acetate/5% mannitol, pH 4.5), liquid gels (e.g. 5% CMC, 1% alginate), or putty pastes (e.g. CMC powder, gelatin powder). Porous hydroxyapatite blocks (discs) are preferred. Also preferred is a putty device comprising collagen matrix powders with Osteonics granules (0.6g CMC: 1.0 g HA). Bone formation was inhibited at low doses (≤ 5 μg), and was equivalent to controls at larger doses (> 10 μg). Also preferred are synthetic devices comprising 50% HA and 50% tricalcium phosphate.

A preferred OP-1/hydroxyapatite (HA) device was prepared by dry mixing OP-1 in a bone collagen device and HA in a ratio of 1:600 (OP-1/HA). The desired ratio of OP-1 to HA was achieved by mixing 2.5 mg OP-1 per gram of matrix, and then adding HA in a ratio of 60:40 (weight per weight) with respect to the matrix. The matrix was a bovine bone matrix, essentially as disclosed in U.S. Patent No. 5,354,557, incorporated by reference herein. The OP-1 was formulated essentially as disclosed in U.S. Patent No. 5,324,819, incorporated by reference herein.

Bilateral Subcutaneous pockets were created in the thorax of rats according to the protocol described in, incorporated by reference herein. A 25 mg aliquot comprising 10 mg of the OP-1 device (25 μg OP-1) was placed into each subcutaneous pocket. The rats were then sacrificed on day 3, 7, 12, 21, or 35. Implants were removed after sacrifice and fixed and demineralized in Boin's solution. The samples were then embedded in paraffin, sectioned and stained with Toluidine Blue.

About 90% of the OP-1/HA composite device comprising an optimal ratio (1:600) of OP-1 to HAC remained at day 7, with significant osteoconversion only on the periphery of the defect site. The time course for HA/OP-1 composite implants revealed a subsequent gradual conversion of the implant to endochondrial bone. Bone growth increased substantially by day 12 (50% of composite remaining) and day 21 (20% of composite remaining). There is no residual HA at day 35 post-implantation, and new bone growth is uniform in the defect site, with no evidence of annulus formation or the formation of any other non-uniform growth patterns.

When the OP-1 device was implanted without HA, the matrix disappeared at about day 21 and good bone and marrow were observed, but there was evidence of annulus formation at the

periphery of the ingrowth. HA disks coated with OP-1 produced no new bone growth after 12 days post-implantation.

The foregoing results demonstrate that HA/OP-1 composite devices incorporating an optimal ratio of osteogenic protein to hydroxyapatite produce uniform ingrowth of new bone, while ensuring complete resorption of HA.

A. Comparison of HA Materials

Two hydroxyapatite ceramic materials obtained from were evaluated as vehicles for delivery of an HA-OP-1 device for bone repair. Both materials were made from the same hydroxyapatite starting material, but one was sintered at 850 degrees Celsius and the other at 800 degrees Celsius. Both materials were in particle form with diameters ranging from about 212 μm to about 425 μm. Each of the hydroxyapatite materials was combined with varying amounts of OP-1 and the composites were evaluated for their ability to stimulate bone formation in rat subcutaneous sites. In general, about 60 mg of these hydroxyapatite occupies the same volume as about 25 mg of collagen.

Subcutaneous implants were made in rats as described above. Generally, bone formed into the implants with 10 μg of OP-1 per 60 mg HA.

The rate of release of OP-1 from collagen and hydroxyapatite into serum was next compared. About 62.5 μg of OP-1 was formulated with 25 mg of collagen or 60 mg of hydroxyapatite in 47.5% ethanol and 0.1% triflouroacetic acid. After lyophilization, the devices were transferred to clean tubes and incubated with 1 ml of serum at 37 degrees Celsius. The serum was removed at the designated time points and replaced with fresh serum. Serum OP-1 levels were quantified by ELISA. It was determined that OP-1 release was similar from both the collagen and hydroxyapatite materials.

B. Effect of Co-Lyophilization of Op-1 and HA

Studies were done to assess the efficacy of implants comprising hydroxyapatite which had been co-lyophilized with OP-1 compared to a non-lyophilized formulation comprising hydroxyapatite particles mixed in an OP-1 solution comprising, in addition to OP-1, 20 mM acetate, pH 4.5, and 5% mannitol.

It was determined that either of the above formulations support adequate bone growth when 5 µg OP-1 was combined in 60 mg hydroxyapatite.

Next, the compatibility of OP-1 and hydroxyapatite particles sterilized by gamma radiation and the stability of an OP-1/HA composite sterilized by gamma radiation were evaluated:

An OP-1/HA device was formulated from 60 mg HA with 60 µg OP-1 by co-lyophilization from 47.5% ethanol/0.01% triflouroacetic acid. The formulation was sterilized using 2.5-3.0 mrads of gamma radiation. A control device (no irradiation) was also made. The irradiated and control devices were eluted with Urea buffer, and analyzed by reverse phase HPLC. Approximately 30-40% of the OP-1 was lost upon irradiation, which is typical of the amount of loss experienced when the collagen device is used. A Ross cell assay indicated that extracted OP-1 retained its biological activity.

V. CAT CRANIAL DEFECT MODEL

An OP-1 bovine collagen device was mixed with hydroxyapatite cement HAC, wherein human osteogenic protein (OP-1) and HAC exist in a ratio of about 1:600 (OP-1: HAC) and was used to repair induced cranial defects in cats. The composite device was essentially the same as the 1:600 device described above in Example 1. An OP-1/ HAC collagen composite device was compared to a control of pure HAC three months after implant using gross examination, computed tomography, and histologic/histometric techniques.

Devices were implanted in the cats as follows. The parietal skull was exposed bilaterally after a midline scalp incision, and the periosteal layer was separated from the skull as a flap. Using a high-speed cutting burr, two full-thickness craniotomies were created on the parietal skull, each about 2.5 cm in diameter, and symmetrical on either side of the midline. In each cat, pure HAC was placed into the area of the right craniotomy defect; whereas the left defect was replaced by the OP-1/HAC collagen device. After shaping of the external contour, implants were allowed to solidify and the incisions were closed in layers.

There were no wound infections, implant infections, or implant extrusions in any cat. The were sacrificed at 3 months post-operative. Skulls were removed and subsequently examined visually for shape, contour and overall appearance. The skulls were then examined by computed tomography with a bone algorithm in order to visualize the implanted areas. Next, the entire

outer calvarial portion of the skull, containing both implanted areas was removed and embedded in methylmethacrylate for undecalcifed whole sections. Paragon- and Von Kossa-stained sections were obtained in order to differentiate HAC, bone, and osteoid components of bone. Histometric analysis was performed to determine the volume fraction of tissue components at the central area of the implant, the implant/bone interface, and the normal calvarial bone. In addition, the percent HAC resorption/replacement was determined by measuring the relative area of remnant implant over the entire implanted area.

A. Gross Histologic Examination

Upon visual examination of the removed skulls, the pure HAC implant appeared intact with shape, contour, and volume very close to that of the initially-applied cement. New bone ingrowth occurred only on the periphery of the defect site. The HAC implant was visible as a lighter color than the surrounding bone and was well-integrated with the surrounding bone. No volume change was apparent in the area of the implant.

In contrast, the OP-1/HAC collagen composite device implant was fully-replaced by new bone. There was no evidence of any remaining implant from the external surface or from the internal surfaces of the calcium. The shape, volume, and contour of the implanted area was preserved in the new bone.

B. Cross-Sectional Imaging

Coronal computerized tomography images of removed skulls revealed full-thickness, stable HAC implant on the HAC (right) side, with some peripheral induction of new bone. On the HAC/OP-1 device side, there was nearly-complete conversion of the composite implant, with the appearance of new lamellar bone. Again, the volume and shape of the new bone was well-preserved.

C. Histology

Paragon and Von Kossa staining revealed growth of new dense bone on the HAC/OP-1 side. The majority of the implant had been replaced by bone. Some remnant implant was seen as dark particles at the center of the implant. By measuring the relative cross-sectional area of the implant versus bone over the entire implanted area, it was calculated that about 93% (SD 3.7) of the composite implant was resorbed and replaced by new bone.

The foregoing data indicate that the composite implant of HAC/OP-1 accelerates the process of implant resorption and new bone formation. Pure HAC was successful only in reconstructing the cranial defect with ostoeintegration on the periphery of the implant site.

VI. USE OF HA/OP-1 DEVICE TO REPAIR BABOON CRANIAL DEFECTS

The efficacy of a single application of various doses of OP-1 absorbed onto sintered hydroxyapatite-based ceramic porous shaped form for osteointegration and bone regeneration in calvarial defects of adult baboons was next determined.

Sintered porous ceramic vehicles were made by heating hydroxyapatite slurries in stages and then sintering. The resulting material was a disc of porous sintered ceramic of about 25 mm in diameter and about 4 mm in thickness. Animals used in the experiments were four clinically-healthy adult male Chacea baboons (*Papio ursinus*). The mean weight of all subjects was 28.7 kg (+/-2.3). Each subject had normal hematologic and biochemical profiles. The animals were obtained from the primate colony at Witwatersrand, Johannesburg, South Africa.

OP-1 was prepared by dissolving 0.0 (5 mM HCl control), 100, or 500 μg OP-1 in 500 μl of 5 mM Hcl. Sintered ceramic discs were prepared as described above. Absorption of the OP-1 into the ceramic carrier was carried out under sterile conditions. The OP-1 solutions were pipetted into the discs and air-dried.

Cranial defects were prepared in each of the animals using a craniotome. Two full-thickness defects, each about 25 mm in diameter, were prepared on each side of the calvaria. The defects were separated by about 3 cm of intervening calvarial bone. In each animal, 2 defects were implanted with sintered discs pretreated with 100 μg OP-1; one defect was treated with a device comprising 500 μg OP-1; and the fourth defect was implanted with a sintered porous disc with no OP-1 (control). Four weeks after implantation, the animals were sacrificed with an intravenous dose of pentobarbitone. Specimen blocks were cut along the sagittal third of the implanted discs and fixed in 10% neutral buffered formaldehyde. Specimens were then decalcified in a formic-hydrochloric acid mixture. Serial sections, each about 5 μm thick, were mounted after recording the position of the anterior and posterior interfaces of the defects with their corresponding calvarial margins. Sections were stained with Goldmer's trichome or with 0.1% toluidine blue in 30% ethanol. A calibrated Zeiss Integration Platte II (Zeiss) with 100 lattice points was used to calculate, by point counting techniques, the fractional volume (in percent) of

the newly-formed bone. Sections were analyzed at a magnification of 40 times with a Zeiss graticule superimposed over five sources selected for histomorphometry and defined as follows: two anterior and posterior interfacial regions (AIF and PIF, respectively) two anterior and posterior internal regions (AIN and PIN, respectively) and a control region (CON). This technique allows the histomorphometric evaluation of the distribution of bone deposition across the hydroxyapatite susbstrata. Each source represented a field of 7.84 mm^2. Morphometry was performed on two sections per specimen, representing parasagittal levels approximately 5 mm apart from each other.

At sacrifice, control specimens showed fibrovascular tissue invasion across the porous spaces of the ceramic device, with some bone formation at the edges of the calvarial defects. There was no bone formation within the central or internal regions of the specimens. No significant resorption of the ceramic occurred in the control.

Porous ceramics pretreated with 100 μg OP-1 showed extensive bone formation within the porous spaces and in direct apposition with the substratum. There was prominent vascular invasion and the newly-formed bone had the features of trabecular woven bone extending into the porous spaces. Moreover, there was complete incorporation of the ceramic disc by newly-formed bone within the severed calvarial bone. Porous ceramics pretreated with 500 μg OP-1 showed bone formation only on the endocranial and pericranial aspects of the specimen, enveloping the ceramic substratum. The internal porous spaces of the ceramics were characterized by the presence of a rich vascular component, but bone formation was not observed. With both the 100 μg and the 500 μg samples, significant resorption of the ceramic material was observed when compared with the control implants. The results of histomorphic analysis of the samples described above are shown in Table 2 below; wherein values are means of four control specimens and 12 OP-1 treated porous HA specimens.

TABLE 2

Treatment	Control	100 μg OP-1	500 μg OP-1
Volume fraction of induced bone (%)	0.7	32.6	23.6

The foregoing results indicate that the use of a ceramic carrier for high concentrations of OP-1 in the absence of a collagenous carrier results in bone formation mainly on the perennial and endocranial aspects of the specimens. With 500 μg OP-1, bone formation mainly enveloped the carrier and was rarely in contact with the substratum. In contrast, use of lower relative amounts of OP-1 (in the range of less than about 1:100 with respect to HAC) resulted in full, uniform induction of new bone.

VII. <u>METAL IMPLANTS FOR GAP DEFECT REPAIR</u>

In other embodiments of the invention, bone ingrowth in a defect is stimulated by implanting a metal implant in a bed of OP devices at the defect site. For example, a defect site, prepared by removing excess or necrotic bone tissue, is filled with an OP device. A metal implant, preferably coated with hydoxyapatite is then placed in the defect so that the OP device fills gap between the metal implant and the edges of the defect site. Preferably, and OP device comprises a morphogen, as herein described, in a suitable adjuvant, or in a collagen matrix, or another matrix as described herein. the following are several examples demonstrating this process.

A. <u>Repair of gap defects using metal implant allograft therapy</u>

Methods of the invention comprise repair of impaired bone stock, in, for example, revision endoprosthetic surgery, using impacted allograft bone chips. Adding the osteoinductive stimulation of osteogenic protein 1 (OP-1) to the osteoconductive effects of the allograft bone chips improves clinical outcome. OP-1 mixed into impacted allograft to improve bone formation and mechanical fixation of hydroxyapatite coated implants.

The effects of the OP-1 in Impacted allograft was evaluated in a canine model. Cylindrical hydroxyapatite-coated titanium alloy implants with an edged surface texture were used. The implants measured 4x9 mm and were inserted unloaded and bilaterally into the proximal humerus of 16 adult mongrel dogs surrounded by a 3 mm gap. Two different doses were tested in 8 animals each: (1) 325 μg OP-1 in 130 mg collagen matrix mixed into 1.3 g of allograft chips. (2) 65 μg OP-1 in 130 mg collagen matrix mixed into 1.3 g of allograft chips. The contralateral humerus were used for control in which the allograft were mixed with collagen matrix without OP-1 only. The dogs were sacrificed after 6 weeks. Bone ingrowth and bone formation in the gap was evaluated by quantitative histomophometry and mechanical fixation of the implants were evaluated by push-out test.

Histomorphometry demonstrated an increase in pan-implant bone volume from 25.5% to 33.5% of total peri-implant gap volume. This increase was found for the 65 μg dose but not for the 325 μg dose. Bone ingrowth was relatively high for control groups (about 35%) and was not altered by OP-1 addition to the allograft. mechanical testing showed that sheer strength, stiffness and energy absorption were not increased for OP-1 simulated groups.

These data study demonstrate that the addition of OP-1 to impacted allograft increases bone formation around the implants using the lowest of the two doses tested. Mechanical fixation of the implants was not improved by OP-1 addition which could be explained by a similar lack of increased bone ingrowth in the groups receiving OP-1. These data indicate a clinical use of OP-1 for stimulation of bone formation in revision endoprosthetic surgery.

B. OP device in prosthetic surgery

In cementless endoprosthetic surgery gaps around implants impair bony fixation and clinical outcome. The following study shows that stimulation of bone healing with osteogenic factors improves clinical outcome of osteogenic prostheses.

Osteogenic protein (OP-1) has previously shown extensive *in vivo* osteoinductive properties in bone defect models, fracture models, and spine fusion models. However, little is known about morphogen's ability to enhance bony fixation of implants. Previously, only TGF-B has demonstrated stimulatory effects on bony fixation and bone formation when applied onto ceramic coated implants surrounded by a gap. The purpose of the present study was to determine if mechanical fixation of uncoated and hydroxyapatite coated implants is enhanced by applying OP-1 in a collagen carrier in a critical-sized, 3 mm, gap around the implants.

Enhancement of implant fixation with recombinant OP-1 was evaluated in a canine model approved by both the Danish and Stanford University control board for animal research. Cylindrical uncoated and hydroxyapatite coated titanium alloy implant (Ti6Al4V) (4x10 mm) with an edged surface texture were used. The implants were inserted unloaded bilaterally into both the medial and the lateral femoral condyles of 26 adult mongrel dogs. All implants were initially surrounded by a 3 mm gap. Eight dogs were included in each implant group. Three different groups for each type of implant were tested. (1) OP-1 device (325 μg OP-1 in 130 mg collagen matrix (2) The collagen matrix without Op-1; (3) Empty gap. The OP-1 device and the collagen carrier was placed in the gap around the implants. The dogs were sacrificed after 8 weeks.

After sacrifice, mechanical fixation of the implants were evaluated by push-out test of a 3.5 mm thick specimen on an Instron universal testing machine. Shear strength, sheer stiffness

and energy absorption was determined. One way ANOVA and paired Students T-test were used. P values less than 0.05 were considered significant.

All dogs completed the study. Mechanical testing showed that shear strength, stiffness and energy absorption were significantly increased for OP-1 stimulated significant increased values above both control and collagen groups with respect to strength and stiffness, whereas for energy absorption OP-1 was only significantly different from the control group. For HA coated implants, the OP-1 group was significantly higher than both the control and collagen groups with respect to stiffness. Whereas for strength and energy absorption the OP-1 group was only significantly different from the control group. Collagen matrix was significantly higher that empty control for both implant types.

The present study demonstrates that the addition of OP-1 device increases mechanical fixation of both uncoated and hydroxyapatite coated implants. The strongest effects were found for uncoated implants where OP-1 device stimulated fixation to the same level as hydroxyapatite coated implants stimulated with the OP-1 device, since the empty control group no fixation was found. Interestingly, the collagen matrix by itself demonstrated a considerable stimulatory effect on fixation, probably due to an osteoconductive effect; whereas the collagen matrix captures stem-cells and other growth factors that will improve bone formation in the gap. The collagen matrix that results also emphasizes the importance of including such carrier controls in studies investigating systems and stimulative agents. For hydroxyapatite coated implants these effects were so dominant that no significant increase was demonstrated for hydroxyapatite coated implants with OP-1 device compared to hydroxyapatite coated implants with the collagen carrier alone, although a 40% increase was found. The study also showed that hydroxyapatite coating alone can bridge a gap of 3 mm in 6 weeks to provide some fixation, but that adding the OP-device strongly enhances bone formation to provide implant fixation in the range of 3 MPa regardless of whether uncoated or HA coated implants are used. These data are interesting for the use of OP-1 for stimulation of bony ingrowth in primary cements endoprosthetic surgery since OP-1 device was shown to enhance mechanical strength for the bone- implant interface of both uncoated and HA coated implants.

C. <u>Hydroxyapatite-coated metal implants</u>

Early incorporation of bone allograft material is of great importance. OP-1 (BMP-7) further enhances bone incorporation around implants, and Pro-Osteon 200 is an alternative to bone allograft.

The purpose of this study was to investigate whether OP-1 device in combination with bone allograft improves the early fixation of implants compared to the allograft alone. We also compared allograft to Pro-Osteon with and without OP-1.

Unloaded cylindrical titanium implants (5-10mm) coated with hydroxyapatite were randomly inserted in the medial and lateral femoral condyles of 6 labrador dogs described in sabelle, et al. Clin Ortho. 274: 283-293 (1992), incorporated by reference herein. A cavity of 11 mm in diameter was created by hand-drilling leaving a gap of 3 mm (0.75cc volume) around the implant. The implant was secured by a footplate and after grafting the gap, a top washer and a screw.

The gaps around the implants were filled according to the following treatment groups: Group 1: Allograft, group 2 Pro-Osteon, group 3: Allograft + OP-1 device, group 4: Pro-Osteon+OP-1 device. Allograft was harvested from a dog not included in the study, frozen at -80°C, thawed and milled in an operating room with laminar air flow. The amount of Pro-Osteon and allograft was standardized by weight. OP-1 was delivered in a device with 2.5 mg recombinant OP-1 in 1 gram bovine type 1 collagen, the concentration of OP-1 in the present study was 300 μg Op-1 in 120 mg collagen carrier. The bone substitute utilized was Pro-Osteon 200 (Interpore, Irvine US) corraline hydroxyapatite granules which has a porous microstructure similar to cortical bone. This product is a non- osteogenic void filler approved by the FDA for metaphyseal defects.

Dogs were sacrificed after 3 weeks and push-out and mechanical fixation was evaluated by a push-out test performed on and Instron Universal testing machine. Ultimate shear strength (MPa), apparent shear stiffness (MPa/mm) and energy absorption (3/mm^2) were determined blindly from load displacement curves.

Statistial data are presented as mean values and standard error of mean. An unpaired t-test was used. (P-values less than 0.05 (two-tailed) were considered significant.) Push-out tests showed, that the OP-1 device enhanced fixation of Pro-Osteon by 900%. No significant differences were found between Pro-Osteon +OP-1 and allograft with or without OP-1, but all three groups had significantly better mechanical parameters compared to the Pro-Osteon group.

Implants treated with bone allograft were much stronger fixated than implants treated with Pro-Osteon alone. However in combination with OP-1, Pro-osteon treated implants were equally fixed compared to the allograft group.

The fact the OP-1 did not improve the fixation of the allograft group indicates that the allograft is a superior gap filling material around non cemented implants compared to Pro-Osteon. on the

other hand, a bone substitute (pro-Osteon) in combination (OP-1) seems to be just as good as bone allograft.

Early incorporation of bank bone allograft is mandatory in revision of anthroplasties. We investigate, if OP-1 (BMP-7) device further enhances bone incorporation around implants and if Pro-Osteon 200 might be an alternative to bone allograft.

24 unloaded cylindrical titanium implants coated with hydroxyapatite were randomly inserted in the femoral condyles of 6 labrador dogs. A 3 mm gap was left around each implant, which were filled according to the following groups: (1) Allograft, (2) Pro-Osteon, (3): Allograft + OP-1, (4) Pro-Osteon + OP-1. the amount of Pro-Osteon, OP-1 and allograft was standardized by weight.

Push out tests after 3 weeks showed, that the OP-1 enhanced fixation of Pro-Osteon by 900%. No significant differences were found between Pro-Osteon + Op-1 and allograft with or without OP-1.

The study showed, that implants treated with bone allograft were much stronger fixated than implants treated with Pro-Osteon alone, however in combination with OP-1, Pro-Osteon treated implants were equally fixed compared to the allograft group.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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(ii) TITLE OF INVENTION: COMPOSITIONS FOR MORPHOGEN-INDUCED OSTEOGENESIS

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

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(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1822 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: HOMO SAPIENS
(F) TISSUE TYPE: HIPPOCAMPUS

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 49..1341
(C) IDENTIFICATION METHOD: experimental
(D) OTHER INFORMATION: /function= "OSTEOGENIC PROTEIN"
/product= "OP1"
/evidence= EXPERIMENTAL
/standard_name= "OP1"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
GGTGCGGGCC CGGAGCCCGG AGCCCGGGTA GCGCGTAGAG CCGGCGCG ATG CAC GTG          57
                                                     Met His Val
                                                       1

CGC TCA CTG CGA GCT GCG GCG CCG CAC AGC TTC GTG GCG CTC TGG GCA          105
Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala Leu Trp Ala
      5                  10                  15

CCC CTG TTC CTG CTG CGC TCC GCC CTG GCC GAC TTC AGC CTG GAC AAC          153
```

Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser Leu Asp Asn
20 25 30 35

GAG GTG CAC TCG AGC TTC ATC CAC CGG CGC CTC CGC AGC CAG GAG CGG 201
Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser Gln Glu Arg
40 45 50

CGG GAG ATG CAG CGC GAG ATC CTC TCC ATT TTG GGC TTG CCC CAC CGC 249
Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu Pro His Arg
55 60 65

CCG CGC CCG CAC CTC CAG GGC AAG CAC AAC TCG GCA CCC ATG TTC ATG 297
Pro Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro Met Phe Met
70 75 80

CTG GAC CTG TAC AAC GCC ATG GCG GTG GAG GAG GGC GGC GGG CCC GGC 345
Leu Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Gly Gly Gly Pro Gly
85 90 95

GGC CAG GGC TTC TCC TAC CCC TAC AAG GCC GTC TTC AGT ACC CAG GGC 393
Gly Gln Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser Thr Gln Gly
100 105 110 115

CCC CCT CTG GCC AGC CTG CAA GAT AGC CAT TTC CTC ACC GAC GCC GAC 441
Pro Pro Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr Asp Ala Asp
120 125 130

ATG GTC ATG AGC TTC GTC AAC CTC GTG GAA CAT GAC AAG GAA TTC TTC 489
Met Val Met Ser Phe Val Asn Leu Val Glu His Asp Lys Glu Phe Phe
135 140 145

CAC CCA CGC TAC CAC CAT CGA GAG TTC CGG TTT GAT CTT TCC AAG ATC 537
His Pro Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu Ser Lys Ile
150 155 160

CCA GAA GGG GAA GCT GTC ACG GCA GCC GAA TTC CGG ATC TAC AAG GAC 585
Pro Glu Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Asp
165 170 175

TAC ATC CGG GAA CGC TTC GAC AAT GAG ACG TTC CGG ATC AGC GTT TAT 633
Tyr Ile Arg Glu Arg Phe Asp Asn Glu Thr Phe Arg Ile Ser Val Tyr
180 185 190 195

CAG GTG CTC CAG GAG CAC TTG GGC AGG GAA TCG GAT CTC TTC CTG CTC 681
Gln Val Leu Gln Glu His Leu Gly Arg Glu Ser Asp Leu Phe Leu Leu
200 205 210

GAC AGC CGT ACC CTC TGG GCC TCG GAG GAG GGC TGG CTG GTG TTT GAC 729
Asp Ser Arg Thr Leu Trp Ala Ser Glu Glu Gly Trp Leu Val Phe Asp
215 220 225

ATC ACA GCC ACC AGC AAC CAC TGG GTG GTC AAT CCG CGG CAC AAC CTG 777
Ile Thr Ala Thr Ser Asn His Trp Val Val Asn Pro Arg His Asn Leu
230 235 240

GGC CTG CAG CTC TCG GTG GAG ACG CTG GAT GGG CAG AGC ATC AAC CCC 825
Gly Leu Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser Ile Asn Pro
245 250 255

AAG TTG GCG GGC CTG ATT GGG CGG CAC GGG CCC CAG AAC AAG CAG CCC 873
Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn Lys Gln Pro
260 265 270 275

TTC ATG GTG GCT TTC TTC AAG GCC ACG GAG GTC CAC TTC CGC AGC ATC 921
Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Phe Arg Ser Ile
280 285 290

CGG TCC ACG GGG AGC AAA CAG CGC AGC CAG AAC CGC TCC AAG ACG CCC 969
Arg Ser Thr Gly Ser Lys Gln Arg Ser Gln Asn Arg Ser Lys Thr Pro
295 300 305

AAG AAC CAG GAA GCC CTG CGG ATG GCC AAC GTG GCA GAG AAC AGC AGC 1017
Lys Asn Gln Glu Ala Leu Arg Met Ala Asn Val Ala Glu Asn Ser Ser
310 315 320

AGC GAC CAG AGG CAG GCC TGT AAG AAG CAC GAG CTG TAT GTC AGC TTC 1065

```
Thr Gln Gly Pro Pro Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr
        115                 120                 125

Asp Ala Asp Met Val Met Ser Phe Val Asn Leu Val Glu His Asp Lys
    130                 135                 140

Glu Phe Phe His Pro Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu
145                 150                 155                     160

Ser Lys Ile Pro Glu Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile
                165                 170                 175

Tyr Lys Asp Tyr Ile Arg Glu Arg Phe Asp Asn Glu Thr Phe Arg Ile
            180                 185                 190

Ser Val Tyr Gln Val Leu Gln Glu His Leu Gly Arg Glu Ser Asp Leu
        195                 200                 205

Phe Leu Leu Asp Ser Arg Thr Leu Trp Ala Ser Glu Glu Gly Trp Leu
    210                 215                 220

Val Phe Asp Ile Thr Ala Thr Ser Asn His Trp Val Val Asn Pro Arg
225                 230                 235                     240

His Asn Leu Gly Leu Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser
                245                 250                 255

Ile Asn Pro Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn
            260                 265                 270

Lys Gln Pro Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Phe
        275                 280                 285

Arg Ser Ile Arg Ser Thr Gly Ser Lys Gln Arg Ser Gln Asn Arg Ser
    290                 295                 300

Lys Thr Pro Lys Asn Gln Glu Ala Leu Arg Met Ala Asn Val Ala Glu
305                 310                 315                     320

Asn Ser Ser Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr
                325                 330                 335

Val Ser Phe Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu
            340                 345                 350

Gly Tyr Ala Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn
        355                 360                 365

Ser Tyr Met Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His
    370                 375                 380

Phe Ile Asn Pro Glu Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln
385                 390                 395                     400

Leu Asn Ala Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile
                405                 410                 415

Leu Lys Lys Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His
            420                 425                 430
```

(2) INFORMATION FOR SEQ ID NO.3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 102 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..102
(D) OTHER INFORMATION: /label= OPX
/note= "wherein each Xaa is independently selected from a group

```
Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe
    325                 330                 335

CGA GAC CTG GGC TGG CAG GAC TGG ATC ATC GCG CCT GAA GGC TAC GCC      1113
Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala
340                 345                 350                 355

GCC TAC TAC TGT GAG GGG GAG TGT GCC TTC CCT CTG AAC TCC TAC ATG      1161
Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met
                360                 365                 370

AAC GCC ACC AAC CAC GCC ATC GTG CAG ACG CTG GTC CAC TTC ATC AAC      1209
Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe Ile Asn
            375                 380                 385

CCG GAA ACG GTG CCC AAG CCC TGC TGT GCG CCC ACG CAG CTC AAT GCC      1257
Pro Glu Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala
        390                 395                 400

ATC TCC GTC CTC TAC TTC GAT GAC AGC TCC AAC GTC ATC CTG AAG AAA      1305
Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys
    405                 410                 415

TAC AGA AAC ATG GTG GTC CGG GCC TGT GGC TGC CAC TAGCTCCTCC           1351
Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His
420                 425                 430

GAGAATTCAG ACCCTTTGGG GCCAAGTTTT TCTGGATCCT CCATTGCTCG CCTTGGCCAG    1411

GAACCAGCAG ACCAACTGCC TTTTGTGAGA CCTTCCCCTC CCTATCCCCA ACTTTAAAGG    1471

TGTGAGAGTA TTAGGAAACA TGAGCAGCAT ATGGCTTTTG ATCAGTTTTT CAGTGGCAGC    1531

ATCCAATGAA CAAGATCCTA CAAGCTGTGC AGGCAAAACC TAGCAGGAAA AAAAAACAAC    1591

GCATAAAGAA AAATGGCCGG GCCAGGTCAT TGGCTGGGAA GTCTCAGCCA TGCACGGACT    1651

CGTTTCCAGA GGTAATTATG AGCGCCTACC AGCCAGGCCA CCCAGCCGTG GGAGGAAGGG    1711

GGCGTGGCAA GGGGTGGGCA CATTGGTGTC TGTGCGAAAG GAAAATTGAC CCGGAAGTTC    1771

CTGTAATAAA TGTCACAATA AAACGAATGA ATGAAAAAAA AAAAAAAAAA A             1822
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 431 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met His Val Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala
1               5                   10                  15

Leu Trp Ala Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser
            20                  25                  30

Leu Asp Asn Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser
        35                  40                  45

Gln Glu Arg Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu
    50                  55                  60

Pro His Arg Pro Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro
65                  70                  75                  80

Met Phe Met Leu Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Gly Gly
                85                  90                  95

Gly Pro Gly Gly Gln Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser
            100                 105                 110
```

of one or more specified amino acids as defined in the specification."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Cys Xaa Xaa His Glu Leu Tyr Val Ser Phe Xaa Asp Leu Gly Trp Xaa
1               5                   10                  15

Asp Trp Xaa Ile Ala Pro Xaa Gly Tyr Xaa Ala Tyr Tyr Cys Glu Gly
            20                  25                  30

Glu Cys Xaa Phe Pro Leu Xaa Ser Xaa Met Asn Ala Thr Asn His Ala
        35                  40                  45

Ile Xaa Gln Xaa Leu Val His Xaa Xaa Xaa Pro Xaa Xaa Val Pro Lys
    50                  55                  60

Xaa Cys Cys Ala Pro Thr Xaa Leu Xaa Ala Xaa Ser Val Leu Tyr Xaa
65                  70                  75                  80

Asp Xaa Ser Xaa Asn Val Ile Leu Xaa Lys Xaa Arg Asn Met Val Val
                85                  90                  95

Xaa Ala Cys Gly Cys His
            100
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 97 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..97
(D) OTHER INFORMATION: /label= Generic-Seq-7
/note= "wherein each Xaa is independently selected from a group of one or more specified amino acids as defined in the specification."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Leu Xaa Xaa Xaa Phe Xaa Xaa Xaa Gly Trp Xaa Xaa Xaa Xaa Xaa Xaa
1               5                   10                  15

Pro Xaa Xaa Xaa Xaa Ala Xaa Tyr Cys Xaa Gly Xaa Cys Xaa Xaa Pro
            20                  25                  30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asn His Ala Xaa Xaa Xaa Xaa Xaa
        35                  40                  45

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Cys Xaa Pro
    50                  55                  60

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa Xaa Xaa
65                  70                  75                  80

Val Xaa Leu Xaa Xaa Xaa Xaa Xaa Met Xaa Val Xaa Xaa Cys Xaa Cys
                85                  90                  95

Xaa
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 102 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..102
(D) OTHER INFORMATION: /label= Generic-Seq-8
/note= "wherein each Xaa is independently selected from a group of one or more specified amino acids as defined in the specification."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
Cys Xaa Xaa Xaa Xaa Leu Xaa Xaa Xaa Phe Xaa Xaa Xaa Gly Trp Xaa
1               5                   10                  15

Xaa Xaa Xaa Xaa Xaa Pro Xaa Xaa Xaa Xaa Ala Xaa Tyr Cys Xaa Gly
            20                  25                  30

Xaa Cys Xaa Xaa Pro Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asn His Ala
        35                  40                  45

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
    50                  55                  60

Xaa Cys Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Leu Xaa Xaa
65                  70                  75                  80

Xaa Xaa Xaa Xaa Xaa Val Xaa Leu Xaa Xaa Xaa Xaa Xaa Met Xaa Val
                85                  90                  95

Xaa Xaa Cys Xaa Cys Xaa
            100
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 97 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..97
(D) OTHER INFORMATION: /label= Generic-Seq-9
/note= "wherein each Xaa is independently selected from a group of one or more specified amino acids as defined in the specification."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1               5                   10                  15

Pro Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Gly Xaa Cys Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
        35                  40                  45

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Pro
    50                  55                  60

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa Xaa Xaa
65                  70                  75                  80

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Cys
                85                  90                  95
```

Xaa

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 102 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..102
(D) OTHER INFORMATION: /label= Generic-Seq-10
/note= "wherein each Xaa is independently selected from a group of one or more specified amino acids as defined in the specification."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1               5                   10                  15

Xaa Xaa Xaa Xaa Xaa Pro Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Gly
            20                  25                  30

Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
        35                  40                  45

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
    50                  55                  60

Xaa Xaa Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Leu Xaa Xaa
65                  70                  75                  80

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
                85                  90                  95

Xaa Xaa Cys Xaa Cys Xaa
            100
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..5
(D) OTHER INFORMATION: /note= "wherein each Xaa is independently selected from a group of one or more specified amino acids as defined in the specification"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Cys Xaa Xaa Xaa Xaa
1               5
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid

(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..5
(D) OTHER INFORMATION: /note= "wherein each Xaa is independently selected from a group of one or more specified amino acids as defined in the specification"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
Cys Xaa Xaa Xaa Xaa
1               5
```

What is claimed is:

1. A method for producing new bone growth at bone defect site in a mammal, the method comprising the step of:

implanting in a defect site a calcium phosphate matrix comprising at least one osteogenic protein.

2. The method of claim 1, wherein said calcium phosphate matrix is a hydroxyapatite matrix.

3. The method of claim 1, wherein said osteogenic protein is a dimeric protein that comprises an amino acid sequence selected from the group consisting of:

(a) a sequence having at least 70% homology with the C-terminal seven-cysteine skeleton of human OP-1, residues 38-139 of SEQ ID NO: 5, and

(b) Generic Sequence 6, SEQ ID NO: 31; and

wherein said morphogen stimulates endochondral bone formation in an in vivo bone assay.

4. The method of claim 1, wherein said osteogenic protein is a dimeric protein that comprises an amino acid sequence selected from the group consisting of:

(a) a sequence having greater than 60% amino acid sequence identity with the C-terminal seven-cysteine skeleton of human OP-1, residues 38-139 of SEQ ID NO: 5, and

(b) OPX sequence defined by SEQ. ID No: 29; and

wherein said osteogenic protein stimulates endochondral bone formation in an in vivo bone assay.

5. The method of claim 1, wherein said osteogenic protein is selected from the group consisting of human OP-1, mouse OP-1, human OP-2, mouse OP-2, 60A, GDF-1, BMP2A, BMP2B, DPP, Vgl, Vgr-1, BMP3, BMP5, and BMP6.

6. The method of claim 1, wherein said osteogenic protein is a conservative substitution variant of a morphogen selected from the group consisting of human OP-1, mouse OP-1,

human OP-2, mouse OP-2, 60A, GDF-1, BMP2A, BMP2B, DPP, Vgl, Vgr-1, BMP3, BMP5, and BMP6.

7. A method for producing new bone growth at a defect site in a mammal, the method comprising the step of:

implanting an osteogenic device in said defect site, the osteogenic device comprising an osteogenic protein and a biocompatible matrix; wherein said biocompatible matrix comprises calcium phosphate in an amount such that the ratio of calcium phosphate to said osteogenic protein is sufficient to produce uniform ingrowth of new bone in said defect site.

8. The method of claim 7, wherein said calcium phosphate is hydroxyapatite.

9. The method of claim 7, wherein said ratio of calcium phosphate to osteogenic protein is less than about 1:10,000.

10. The method of claim 7, wherein said ratio of calcium phosphate to osteogenic protein is about 1:2000.

11. The method of claim 7, wherein said ratio is about 1:600.

12. The method according to claim 7, wherein said ratio is 1:700.

13. The method according to claim 7, wherein said ratio is 1:500.

14. The method according to claim 7, wherein said ratio is 1:1000.

15. A method for stimulating new bone growth at a defect site in a mammalian bone, comprising the step of:

introducing calcium phosphate and osteogenic protein to a defect site in a ratio sufficient to produce uniform ingrowth of new bone.

16. The method of claim 15, wherein said calcium phosphate is hydroxyapatite.

17. The method of claim 16, wherein said hydroxyapatite is a sintered hydroxyapatite.

18. The method of claim 15, wherein said ratio is less than about 1:100.

19. The method of claim 15, wherein said ratio is about 1:500

20. The method of claim 15, wherein said ratio is about 1:600.

21. The method of claim 15, wherein said ratio is about 1:700.

22. A method for inducing uniform calcium resorption in a bone defect site, the method comprising the steps of:
implanting in said defect site an osteogenic device comprising a biocompatible matrix, calcium phosphate, and an osteogenic protein; wherein said osteogenic protein and said calcium phosphate are present in said device in a ratio of less than about 1:1000.

23. The method of claim 22, wherein said calcium phosphate is hydroxyapatite.

24. The method of claim 22, wherein said ratio is about 1:500.

25. The method of claim 22, wherein said ratio is about 1:600.

26. The method of claim 22, wherein said ratio is about 1:700.

27. A method for promoting bone ingrowth in a defect site, the method comprising the steps of:
(a) implanting in a defect site a metal implant; and
(b) surrounding said implant with a composition comprising a morphogen selected for the groups consisting of:
(1) a morphogen having at least 70% amino acid homology with the C-terminal, seven-cysteine domain of human OP-1, SEQ ID NO: and
(2) a morphogen having at least 60% amino acid identity with the C-terminal, seven-cysteine domain of human OP-1, SEQ ID NO: .

28. The method of claim 27, wherein said metal implant is a titanium implant.

29. The method of claim 27, wherein said metal implant is coated with hydroxyapatite.

30. The method of claim 27, wherein said morphogen is OP-1.

31. The method of claim 27, wherein said morphogen is selected from the group consists of BMP2, BMP4, BMP5, and BMP6.

32. The method of claim 27, wherein said composition further comprises a collagen matrix.

33. The method of claim 27, wherein said composition further comprises a carboxy methyl cellulose matrix.

34. The method of claim 27 wherein said composition further comprises a calcium phosphate matrix.

35. The method of claim 34, wherein said calcium phosphate matrix is a hydoxyapatite matrix.

hOP-1	Cys	Lys	Lys	His	Glu	Leu	Tyr	Val
mOP-1	...	...	...	...	...	...	...	...
hOP-2	...	Arg	Arg	...	...	...	...	...
mOP-2	...	Arg	Arg	...	...	...	...	...
mOP-3	...	Arg	Arg	...	...	...	...	...
DPP	...	Arg	Arg	...	Ser	...	...	...
Vgl	...	...	Lys	Arg	His	...	...	...
Vgr-1	...	...	...	...	Gly	...	...	...
CBMP-2A	...	...	Arg	...	Pro	...	...	...
CBMP-2B	...	Arg	Arg	...	Ser	...	...	...
BMP3	...	Ala	Arg	Arg	Tyr	...	Lys	...
GDF-1	...	Arg	Ala	Arg	Arg	...	...	...
60A	...	Gln	Met	Glu	Thr	...	...	...
BMP5	...	...	...	...	...	...	...	...
BMP6	...	Arg	...	...	...	...	...	...
	1				5			

FIG. 1A

hOP-1	Ser	Phe	Arg	Asp	Leu	Gly	Trp	Gln	Asp
mOP-1	...	...	...	...	...	...	...	...	...
hOP-2	...	...	Gln	...	...	...	...	Leu	...
mOP-2	Ser	...	...	...	...	...	...	Leu	...
mOP-3	...	...	...	...	...	...	...	Leu	...
DPP	Asp	...	Ser	...	Val	...	...	Asp	...
Vg1	Glu	...	Lys	...	Val	...	...	...	Asn
Vgr-1	...	...	Gln	...	Val	...	...	...	...
CBMP-2A	Asp	...	Ser	...	Val	...	...	Asn	...
CBMP-2B	Asp	...	Ser	...	Val	...	...	Asn	...
BMP3	Asp	...	Ala	...	Ile	...	...	Ser	Glu
GDF-1	...	...	...	Glu	Val	...	...	His	Arg
60A	Asp	...	Lys	...	...	...	...	His	...
BMP5	...	...	...	...	...	...	...	...	...
BMP6	...	...	Gln	...	...	...	...	...	...
		10					15		

FIG. 1B

hOP-1	Trp	Ile	Ile	Ala	Pro	Glu	Gly	Tyr	Ala
mOP-1	...	...	...	...	...	...	...	...	...
hOP-2	...	Val	...	...	...	Gln	...	...	Ser
mOP-2	...	Val	...	...	...	Gln	...	...	Ser
mOP-3	Ser	Val	...	...	...	Gln	...	...	Ser
DPP	...	...	Val	...	...	Leu	...	...	Asp
Vgl	...	Val	...	...	...	Gln	...	...	Met
Vgr-1	...	...	...	...	...	Lys	...	...	...
CBMP-2A	...	...	Val	...	...	Pro	...	...	His
CBMP-2B	...	...	Val	...	...	Pro	...	...	Gln
BMP3	...	...	...	Ser	...	Lys	Ser	Phe	Asp
GDF-1	...	Val	...	...	...	Arg	...	Phe	Leu
60A	...	...	...	...	...	...	...	...	Gly
BMP5	...	...	...	...	...	...	...	...	...
BMP6	...	...	...	...	...	Lys	...	...	...
			20					25	

FIG. 1C

hOP-1	Ala	Tyr	Tyr	Cys	Glu	Gly	Glu	Cys	Ala
mOP-1	...	...	...	...	...	...	...	...	...
hOP-2	...	...	...	...	...	...	...	...	Ser
mOP-2	...	...	...	...	...	...	...	...	...
mOP-3	...	...	...	...	Ala	...	...	...	Ile
DPP	...	...	...	...	His	...	Lys	...	Pro
Vgl	...	Asn	...	...	Tyr	...	...	...	Pro
Vgr-1	...	Asn	...	...	Asp	...	...	...	Ser
CBMP-2A	...	Phe	...	...	His	...	Glu	...	Pro
CBMP-2B	...	Phe	...	...	His	...	Asp	...	Pro
BMP3	...	...	...	...	Ser	...	Ala	...	Gln
GDF-1	...	Asn	...	...	Gln	...	Gln	...	...
60A	...	Phe	...	...	Ser	...	...	...	Asn
BMP5	...	Phe	...	...	Asp	...	...	...	Ser
BMP6	...	Asn	...	...	Asp	...	...	...	Ser
				30					35

FIG. 1D

hOP-1	Phe	Pro	Leu	Asn	Ser	Tyr	Met	Asn	Ala
mOP-1	...	...	...	...	...	...	...	...	...
hOP-2	...	...	...	Asp	...	Cys	...	...	...
mOP-2	...	...	...	Asp	...	Cys	...	...	...
mOP-3	Tyr	...	...	...	...	Cys	...	...	Ser
DPP	...	...	...	Ala	Asp	His	Phe	...	Ser
Vgl	Tyr	...	...	Thr	Glu	Ile	Leu	...	Gly
Vgr-1	...	...	...	...	Ala	His	...	...	...
CBMP-2A	...	...	...	Ala	Asp	His	Leu	...	Ser
CBMP-2B	...	...	...	Ala	Asp	His	Leu	...	Ser
GDF-1	Leu	...	Val	Ala	Leu	Ser	Gly	Ser**	...
BMP3	...	...	Met	Pro	Lys	Ser	Leu	Lys	Pro
60A	...	...	...	...	Ala	His	...	...	...
BMP5	...	...	...	...	Ala	His	Met	...	...
BMP6	...	...	...	...	Ala	His	Met	...	...
					40				

FIG. 1E

hOP-1	Thr	Asn	His	Ala	Ile	Val	Gln	Thr	Leu
mOP-1	...	...	...	...	...	...	...	...	...
hOP-2	...	...	...	...	...	Leu	...	Ser	...
mOP-2	...	...	...	...	...	Leu	...	Ser	...
mOP-3	...	...	...	...	Thr	Met	...	Ala	...
DPP	...	...	...	...	Val	...	...	...	...
Vgl	Ser	...	...	...	...	Leu	...	...	...
Vgr-1	...	...	...	...	...	...	...	...	...
CBMP-2A	...	...	...	...	...	...	...	...	...
CBMP-2B	...	...	...	...	...	...	...	...	...
BMP3	Ser	...	...	...	Thr	Ile	...	Ser	Ile
GDF-1	Leu	...	...	...	Val	Leu	Arg	Ala	...
60A	...	...	...	...	...	...	...	...	...
BMP5	...	...	...	...	...	...	...	...	...
BMP6	...	...	...	...	...	...	...	...	...
	45					50			

FIG. 1F

hOP-1	Val	His	Phe	Ile	Asn	Pro	Glu	Thr	Val
mOP-1	...	...	...	...	...	...	Asp	...	...
hOP-2	...	His	Leu	Met	Lys	...	Asn	Ala	...
mOP-2	...	His	Leu	Met	Lys	...	Asp	Val	...
mOP-3	...	...	Leu	Met	Lys	...	Asp	Ile	Ile
DPP	...	Asn	Asn	Asn	...	...	Gly	Lys	...
Vgl	...	...	Ser	...	Glu	...	...	Asp	Ile
Vgr-1	...	...	Val	Met	...	...	...	Tyr	...
CBMP-2A	...	Asn	Ser	Val	...	Ser	...	Lys	Ile
CBMP-2B	...	Asn	Ser	Val	...	Ser	...	Ser	Ile
BMP3	...	Arg	Ala**	Gly	Val	Val	Pro	Gly	Ile
GDF-1	Met	...	Ala	Ala	Ala	...	Gly	Ala	Ala
60A	...	...	Leu	Leu	Glu	...	Lys	Lys	...
BMP5	...	...	Leu	Met	Phe	...	Asp	His	...
BMP6	...	...	Leu	Met	...	...	...	Tyr	...
		55					60		

FIG. 1G

hOP-1	Pro	Lys	Pro	Cys	Cys	Ala	Pro	Thr	Gln
mOP-1	...	...	...	...	...	...	...	...	...
hOP-2	...	...	Ala	...	...	...	...	...	Lys
mOP-2	...	...	Ala	...	...	...	...	...	Lys
mOP-3	...	...	Val	...	...	Val	...	...	Glu
DPP	...	...	Ala	...	...	Val	...	...	...
Vgl	...	Leu	...	...	...	Val	...	...	Lys
Vgr-1	...	...	...	...	...	...	...	...	Lys
CBMP-2A	...	...	Ala	...	...	Val	...	...	Glu
CBMP-2B	...	...	Ala	...	...	Val	...	...	Glu
BMP3	...	Glu	...	...	...	Val	...	Glu	Lys
GDF-1	Asp	Leu	...	...	...	Val	...	Ala	Arg
60A	...	...	...	...	...	...	...	...	Arg
BMP5	...	...	...	...	...	...	...	...	Lys
BMP6	...	...	...	...	...	...	...	...	Lys
			65					70	

FIG. 1H

hOP-1	Leu	Asn	Ala	Ile	Ser	Val	Leu	Tyr	Phe
mOP-1	...	...	...	...	...	...	...	...	...
hOP-2	...	Ser	...	Thr	...	...	...	...	Tyr
mOP-2	...	Ser	...	Thr	...	...	...	...	Tyr
mOP-3	...	Ser	...	...	...	Leu	...	...	Tyr
Vgl	Met	Ser	Pro	...	...	Met	...	Phe	Tyr
Vgr-1	Val	...	...	...	...	...	...	...	...
DPP	...	Asp	Ser	Val	Ala	Met	...	...	Leu
CBMP-2A	...	Ser	...	...	...	Met	...	...	Leu
CBMP-2B	...	Ser	...	...	...	Met	...	...	Leu
BMP3	Met	Ser	Ser	Leu	...	Ile	...	Phe	Tyr
GDF-1	...	Ser	Pro	...	...	...	...	Phe	...
60A	...	Gly	...	Leu	Pro	...	...	...	His
BMP5	...	...	...	...	...	...	...	...	...
BMP6	...	...	...	...	...	...	...	...	...
				75					80

FIG. 1I

hOP-1	Asp	Asp	Ser	Ser	Asn	Val	Ile	Leu	Lys
mOP-1	...	...	...	...	...	...	...	...	...
hOP-2	...	Ser	...	Asn	...	...	...	...	Arg
mOP-2	...	Ser	...	Asn	...	...	...	...	Arg
mOP-3	...	Arg	Asn	Asn	...	...	...	...	Arg
DPP	Asn	...	Gln	...	Thr	...	Val	...	...
Vgl	...	Asn	Asn	Asp	...	...	Val	...	Arg
Vgr-1	...	...	Asn	...	...	...	...	...	...
CBMP-2A	...	Glu	Asn	Glu	Lys	...	Val	...	...
CBMP-2B	...	Glu	Tyr	Asp	Lys	...	Val	...	...
BMP3	...	Glu	Asn	Lys	...	...	Val	...	...
GDF-1	...	Asn	...	Asp	...	...	Val	...	Arg
60A	Leu	Asn	Asp	Glu	...	...	Asn	...	...
BMP5	...	...	...	...	...	...	...	...	...
BMP6	...	...	Asn	...	...	...	...	...	...
					85				

FIG. 1J

hOP-1	Lys	Tyr	Arg	Asn	Met	Val	Val	Arg
mOP-1	...	...	...	...	...	...	...	...
hOP-2	...	His	...	...	...	...	...	Lys
mOP-2	...	His	...	...	...	...	...	Lys
mOP-3	Arg	Glu	...	...	...	...	...	Gln
DPP	Asn	...	Gln	Glu	...	Thr	...	Val
Vgl	His	...	Glu	...	...	Ala	...	Asp
Vgr-1	...	...	...	...	...	...	...	...
CBMP-2A	Asn	...	Gln	Asp	...	...	...	Glu
CBMP-2B	Asn	...	Gln	Glu	...	...	...	Glu
BMP3	Val	...	Pro	...	...	Thr	...	Glu
GDF-1	Gln	...	Glu	Asp	...	...	...	Asp
60A	...	...	...	...	...	Ile	...	Lys
BMP5	...	...	...	...	...	...	...	...
BMP6	...	...	...	TrP	...	...	...	...
	90					95		

FIG. 1K

hOP-1	Ala	Cys	Gly	Cys	His
mOP-1	...	...	...	...	...
hOP-2	...	...	...	...	...
mOP-2	...	...	...	...	...
mOP-3	...	...	...	...	...
DPP	Gly	...	...	...	Arg
Vgl	Glu	...	...	...	Arg
Vgr-1	...	...	...	...	...
CBMP-2A	Gly	...	...	...	Arg
CBMP-2B	Gly	...	...	...	Arg
BMP3	Ser	...	Ala	...	Arg
GDF-1	Glu	...	...	...	Arg
60A	Ser	...	...	...	...
BMP5	Ser	...	...	...	...
BMP6	...	...	...	...	...
			100		

FIG. 1L

**Between residues 56 and 57 of BMP3 is a Val residue; between residues 43 and 44 of GDF-1 lies the amino acid sequence Gly-Gly-Pro-Pro.

FIG. 1M

AMINO ACID POSITION			Xaa=
SEQ. ID NO: 4	SEQ ID NO: 5	SEQ ID NO: 8	
	2	2	Lys, Arg, Ala, or Gln
	3	3	Lys, Arg, or Met
	4	4	His, Arg, or Gln
	5	5	Glu, Ser, His, Gly, Arg, Pro, Thr, or Tyr
2	7		Tyr or Lys
3	8		Val or Ile
4	9		Ser, Asp, or Glu
6	11		Arg, Gln, Ser, Lys, or Ala
7	12		Asp or Glu
8	13		Leu, Val, or Ile
11	16		Gln, Leu, Asp, His, Asn, or Ser
12	17		Asp, Arg, Asn, or Glu
13	18		Trp or Ser
14	19		Ile or Val
15	20		Ile or Val
16	21		Ala or Ser
18	23		Glu, Gln, Leu, Lys, Pro, or Arg
19	24		Gly or Ser
20	25		Tyr or Phe
21	26		Ala, Ser, Asp, Met, His, Gln, Leu, or Gly
23	28		Tyr, Asn, or Phe
26	31		Glu, His, Tyr, Asp, Gln, Ala, or Ser
28	33		Glu, Lys, Asp, Gln, or Ala
30	35		Ala, Ser, Pro, Gln, Ile, or Asn
31	36		Phe, Leu, or Tyr
33	38		Leu, Val, or Met
34	39		Asn, Asp, Ala, Thr, or Pro
35	40		Ser, Asp, Glu, Leu, Ala, or Lys
36	41		Tyr, Cys, His, Ser, or Ile
37	42		Met, Phe, Gly, or Leu
38	43		Asn, Ser, or Lys
39	44		Ala, Ser, Gly, or Pro
40	45		Thr, Leu, or Ser
44	49		Ile, Val, or Thr
45	50		Val, Leu, Met, or Ile
46	51		Gln or Arg
47	52		Thr, Ala, or Ser
48	53		Leu or Ile
49	54		Val or Met
50	55		His, Asn, or Arg
51	56		Phe, Leu, Asn, Ser, Ala, or Val
52	57		Ile, Met, Asn, Ala, Val, Gly, or Leu
53	58		Asn, Lys, Ala, Glu, Gly, or Phe
54	59		Pro, Ser, or Val

FIG. 2A

AMINO ACID POSITION			
SEQ. ID NO: 4	SEQ ID NO: 5	SEQ ID NO: 8	Xaa=
55	60		Glu, Asp, Asn, Gly, Val, Pro, or Lys
56	61		Thr, Ala, Val, Lys, Asp, Tyr, Ser, Gly, Ile, or His
57	62		Val, Ala, or Ile
58	63		Pro or Asp
59	64		Lys, Leu, or Glu
60	65		Pro, Val, or Ala
63	68		Ala or Val
65	70		Thr, Ala, or Glu
66	71		Gln, Lys, Arg, or Glu
67	72		Leu, Met, or Val
68	73		Asn, Ser, Asp, or Gly
69	74		Ala, Pro, or Ser
70	75		Ile, Thr, Val, or Leu
71	76		Ser, Ala, or Pro
72	77		Val, Leu, Met, or Ile
74	79		Tyr or Phe
75	80		Phe, Tyr, Leu, or His
76	81		Asp, Asn, or Leu
77	82		Asp, Glu, Asn, Arg, or Ser
78	83		Ser, Gln, Asn, Tyr, or Asp
79	84		Ser, Asn, Asp, Glu, or Lys
80	85		Asn, Thr, or Lys
82	87		Ile, Val, or Asn
84	89		Lys or Arg
85	90		Lys, Asn, Gln, His, Arg, or Val
86	91		Tyr, Glu, or His
87	92		Arg, Gln, Glu, or Pro
88	93		Asn, Glu, Trp, or Asp
90	95		Val, Thr, Ala, or Ile
92	97		Arg, Lys, Val, Asp, Gln, or Glu
93	98		Ala, Gly, Glu, or Ser
95	100		Gly or Ala
97	102		His or Arg

FIG. 2B

AMINO ACID POSITION			
SEQ ID NO: 6	SEQ ID NO: 7	SEQ ID NO: 9	Xaa=
	2	2	Lys, Arg, Gln, Ser, His, Glu, Ala, or Cys
	3	3	Lys, Arg, Met, Thr, Leu, Tyr, or Ala
	4	4	His, Gln, Arg, Lys, Thr, Leu, Val, Pro, or Tyr
	5	5	Gln, Thr, His, Arg, Pro, Ser, Ala, Asn, Tyr, Lys, Asp, or Leu
1	6		Phe, Leu, or Glu
2	7		Tyr, Phe, His, Arg, Thr, Lys, Gln, Val, or Glu
3	8		Val, Ile, Leu, or Asp
4	9		Ser, Asp, Glu, Asn, or Phe
5	10		Phe or Glu
6	11		Arg, Gln, Lys, Ser, Glu, Ala, or Asn
7	12		Asp, Glu, Leu, Ala, or Gln
8	13		Leu, Val, Met, Ile, or Phe
9	14		Gly, His, or Lys
10	15		Trp or Met
11	16		Gln, Leu, His, Glu, Asn, Asp, Ser, or Gly
12	17		Asp, Asn, Ser, Lys, Arg, Glu, or His
13	18		Trp or Ser
14	19		Ile or Val
15	20		Ile or Val
16	21		Ala, Ser, Tyr, or Trp
18	23		Glu, Lys, Gln, Met, Pro, Leu, Arg, His, or Lys
19	24		Gly, Glu, Asp, Lys, Ser, Gln, Arg, or Phe
20	25		Tyr or Phe
21	26		Ala, Ser, Gly, Met, Gln, His, Glu, Asp, Leu, Asn, Lys, or Thr
22	27		Ala or Pro
23	28		Tyr, Phe, Asn, Ala, or Arg
24	29		Tyr, His, Glu, Phe, or Arg
26	31		Glu, Asp, Ala, Ser, Tyr, His, Lys, Arg, Gln, or Gly
28	33		Glu, Asp, Leu, Val, Lys, Gly, Thr, Ala, or Gln
30	35		Ala, Ser, Ile, Asn, Pro, Glu, Asp, Phe, Gln, or Leu
31	36		Phe, Tyr, Leu, Asn, Gly, or Arg
32	37		Pro, Ser, Ala, or Val
33	38		Leu, Met, Glu, Phe, or Val
34	39		Asn, Asp, Thr, Gly, Ala, Arg, Leu, or Pro
35	40		Ser, Ala, Glu, Asp, Thr, Leu, Lys, Gln, or His
36	41		Tyr, His, Cys, Ile, Arg, Asp, Asn, Lys, Ser, Glu, or Gly
37	42		Met, Leu, Phe, Val, Gly, or Tyr
38	43		Asn, Glu, Thr, Pro, Lys, His, Gly, Met, Val, or Arg
39	44		Ala, Ser, Gly, Pro, or Phe
40	45		Thr, Ser, Leu, Pro, His, or Met
41	46		Asn, Lys, Val, Thr, or Gln
42	47		His, Tyr, or Lys

FIG. 3A

AMINO ACID POSITION			
SEQ ID NO: 6	SEQ ID NO: 7	SEQ ID NO: 9	Xaa=
43	48		Ala, Thr, Leu, or Tyr
44	49		Ile, Thr, Val, Phe, Tyr, Met, or Pro
45	50		Val, Leu, Met, Ile, or His
46	51		Gln, Arg, or Thr
47	52		Thr, Ser, Ala, Asn, or His
48	53		Leu, Asn, or Ile
49	54		Val, Met, Leu, Pro, or Ile
50	55		His, Asn, Arg, Lys, Tyr, or Gln
51	56		Phe, Leu, Ser, Asn, Met, Ala, Arg, Glu, Gly, or Gln
52	57		Ile, Met, Leu, Val, Lys, Gln, Ala, or Tyr
53	58		Asn, Phe, Lys, Glu, Asp, Ala, Gln, Gly, Leu, or Val
54	59		Pro, Asn, Ser, Val, or Asp
55	60		Glu, Asp, Asn, Lys, Arg, Ser, Gly, Thr, Gln, Pro, or His
56	61		Thr, His, Tyr, Ala, Ile, Lys, Asp, Ser, Gly, or Arg
57	62		Val, Ile, Thr, Ala, Leu, or Ser
58	63		Pro, Gly, Ser, Asp, or Ala
59	64		Lys, Leu, Pro, Ala, Ser, Glu, Arg, or Gly
60	65		Pro, Ala, Val, Thr, or Ser
61	66		Cys, Val, or Ser
63	68		Ala, Val, or Thr
65	70		Thr, Ala, Glu, Val, Gly, Asp, or Tyr
66	71		Gln, Lys, Glu, Arg, or Val
67	72		Leu, Met, Thr, or Tyr
68	73		Asn, Ser, Gly, Thr, Asp, Glu, Lys, or Val
69	74		Ala, Pro, Gly, or Ser
70	75		Ile, Thr, Leu, or Val
71	76		Ser, Pro, Ala, Thr, Asn, or Gly
72	77		Val, Ile, Leu, or Met
74	79		Tyr, Phe, Arg, Thr, or Met
75	80		Phe, Tyr, His, Leu, Ile, Lys, Gln, or Val
76	81		Asp, Leu, Asn, or Glu
77	82		Asp, Ser, Arg, Asn, Glu, Ala, Lys, Gly, or Pro
78	83		Ser, Asn, Asp, Tyr, Ala, Gly, Gln, Met, Glu, or Lys
79	84		Ser, Asn, Glu, Asp, Val, Lys, Gly, Gln, or Arg
80	85		Asn, Lys, Thr, Pro, Val, Ile, Arg, Ser, or Gln
81	86		Val, Ile, Thr, or Ala
82	87		Ile, Asn, Val, Leu, Tyr, Asp, or Ala
83	88		Leu, Tyr, Lys, or Ile
84	89		Lys, Arg, Asn, Tyr, Phe, Thr, Glu, or Gly
85	90		Lys, Arg, His, Gln, Asn, Glu, or Val
86	91		Tyr, His, Glu, or Ile
87	92		Arg, Glu, Gln, Pro, or Lys
88	93		Asn, Asp, Ala, Glu, Gly, or Lys

FIG. 3B

AMINO ACID POSITION			
SEQ ID NO: 6	SEQ ID NO: 7	SEQ ID NO: 9	Xaa=
89	91		Met or Ala
90	95		Val, Ile, Ala, Thr, Ser, or Lys
91	96		Val or Ala
92	97		Arg, Lys, Gln, Asp, Glu, Val, Ala, Ser, or Thr
93	98		Ala, Ser, Glu, Gly, Arg, or Thr
95	100		Gly, Ala, or Thr
97	102		His, Arg, Gly, Leu, or Ser

FIG. 3C

AMINO ACID POSITION	
SEQ ID NO: 3	Xaa=
2	Lys or Arg
3	Lys or Arg
11	Arg or Gln
16	Gln or Leu
19	Ile or Val
23	Glu or Gln
26	Ala or Ser
35	Ala or Ser
39	Asn or Asp
41	Tyr or Cys
50	Val or Leu
52	Ser or Thr
56	Phe or Leu
57	Ile or Met
58	Asn or Lys
60	Glu, Asp, or Asn
61	Thr, Ala, or Val
65	Pro or Ala
71	Gln or Lys
73	Asn or Ser
75	Ile or Thr
80	Phe or Tyr
82	Asp or Ser
84	Ser or Asn
89	Lys or Arg
91	Tyr or His
97	Arg or Lys

FIG. 4

PCT
WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
A61L 27/00, C07K 14/51

A3

(11) International Publication Number: WO 98/51354

(43) International Publication Date: 19 November 1998 (19.11.98)

(21) International Application Number: PCT/US98/09951

(22) International Filing Date: 15 May 1998 (15.05.98)

(30) Priority Data:

60/046,589	15 May 1997 (15.05.97)	US
09/039,107	14 March 1998 (14.03.98)	US

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(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published

With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(88) Date of publication of the international search report: 11 March 1999 (11.03.99)

(54) Title: COMPOSITIONS FOR MORPHOGEN-INDUCED OSTEOGENESIS

(57) Abstract

Disclosed herein are improved osteogenic devices and methods of use thereof for repair of bone and cartilage defects.

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INTERNATIONAL SEARCH REPORT

Inte ional Application No
PCT/US 98/09951

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61L27/00 C07K14/51

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

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C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 91 11148 A (ANDREASSEN TROELS TORP ;JOERGENSEN JOERGEN PETER HOLMB (DK); BAK B) 8 August 1991 see claims	1,2,5-21
X	WO 93 25246 A (STRYKER CORP) 23 December 1993 see claims	1,2,5-21
X	EP 0 361 896 A (COLLAGEN CORP) 4 April 1990 see claims; examples	1,2,7,8
X	EP 0 616 814 A (SQUIBB BRISTOL MYERS CO) 28 September 1994 see claims	1,2,7,8

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 January 1999	22/01/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	ESPINOSA, M

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Int. ...ional Application No
PCT/US 98/09951

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	WO 93 00049 A (XOMA CORP) 7 January 1993 see claims	1,2,7,8
X	WO 96 40297 A (STRYKER CORP) 19 December 1996 see claims; examples	1,2
X	WO 95 33502 A (CREATIVE BIOMOLECULES INC) 14 December 1995 see claims	1,2
A	WO 94 10203 A (CREATIVE BIOMOLECULES INC) 11 May 1994 see claims	1,2
A	EP 0 309 241 A (COLLAGEN CORP) 29 March 1989	
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...ernational application No.

PCT/US 98/09951

INTERNATIONAL SEARCH REPORT

Box I Observations where certain claims were found unsearchable (Continuation f item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 1-35
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 1-35 (are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

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